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Begin the article with a short summary.

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Submit each table on a separate page, typed double spaced. Please use standard abbreviations; otherwise spell out.

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Contents

Cancer Treatment Reports
Vol. 60, No. 3
March 1976

LETTERS Page

Results of Butocin (NSC-172755) Testing Against Tumors in Animals 215
John M. Venditti
National Cancer Institute, Bethesda, Maryland

Myelotoxicity and Orthostatic Hypotension of Cyclocytidine (NSC-145668) 215
Robert A. Minow, Jeffrey A. Gottlieb, Robert M. O'Bryan, and Barth Hoogstraten
The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston

Levamisole (NSC-177023) in Clinical Immunotherapy 217
Willem K. Amery
Janssen Pharmaceutica, Beerse, Belgium

Selection of Carriers for Alkylating Moieties to Increase Their Antitumor Specificity 217
Susan M. Sieber and Richard H. Adamson
National Cancer Institute, Bethesda, Maryland

DTIC (NSC-45388) Toxicity 219
George E. Moore and Donna Meiselbaugh
Denver General Hospital, Colorado

Calusterone (NSC-88536) in Advanced Breast Cancer 220
G. Falkson and H.C. Falkson
H.F. Verwoerd Hospital and University of Pretoria, South Africa

Prolonged Survival of Patients With Extensive Oat Cell Carcinoma Treated With Radiotherapy and Cyclophosphamide (NSC-26271), Vincristine (NSC-67574), and Methotrexate (NSC-740) 221
Jacob Bitran, Harvey Golomb, Richard K. Desser, and Martin Colman
The University of Chicago Cancer Research Center, and the Franklin McLean Memorial Research Institute, Illinois

Prevention by Coenzyme Q_{10} (NSC-140865) of the Inhibition by Adriamycin (NSC-123127) of Coenzyme Q_{10} Enzymes 223
Takeo Kishi and Karl Folkers
The University of Texas at Austin

ARTICLES

Laser Differential Light-Scattering Bioassay for Methotrexate (NSC-740) 225
Philip J. Wyatt, Robert F. Pittillo, Louise S. Rice, Carolyn Woolley, and L. B. Mellett
Southern Research Institute, Birmingham, Alabama

Methyl-CCNU (NSC-95441) in Advanced Colorectal Carcinoma After Failure of 5-Fluorouracil (NSC-19893) Therapy 235
B. J. Cedermark, M. S. Didolkar, and E. G. Elias
Roswell Park Memorial Institute, Buffalo

Page

Vincristine (NSC-67574) in the Treatment of Small-Cell Anaplastic Carcinoma of the Lung 239
Per Dombernowsky, Heine H. Hansen, Peter G. Sørensen, and Bo Hainau
The Finsen Institute and Bispebjerg Hospital, Copenhagen, Denmark

BCNU (NSC-409962) and Procarbazine (NSC-77213) Treatment for Malignant Brain Tumors 243
V. A. Levin, D. C. Crafts, C. B. Wilson, M. J. Schultz, E. B. Boldrey, K. J. Enot, T. L. Pischer, M. Seager, and R. M. Elashoff
University of California Medical School, San Francisco

Response to Medroxyprogesterone Acetate (NSC-26386) as Secondary Hormone Therapy for Metastatic Breast Cancer in Postmenopausal Women 251
David J. Klaassen, Edna F. Rapp, and Wolfgang E. Hirte
Ottawa Civic Clinic, Ontario Cancer Foundation, and University of Ottawa, Canada

BRIEF REPORTS AND PRELIMINARY COMMUNICATIONS

Comparative Experimental Evaluation of Immunodepressive and Toxic Effects of L-Asparaginase (NSC-109229) From *Escherichia coli* and From *Erwinia carotovora* 255
M. Cavanna, G. Celle, M. Dodero, A. Picciotto, I. Pannacciulli, and G. Brambilla
Istituto di Farmacologia, Università di Genova, Italy

Dose Fractionation Does Not Prevent Repair of Potentially Lethal Damage Induced by Bleomycin (NSC-125066) In Vivo 259
Peter R. Twentyman
Medical Research Council, Cambridge, England

In Vivo Effects of Phenylalanine Ammonia-Lyase 261
Joseph Roberts, Franz A. Schmid, and Katsuji Takai
Sloan-Kettering Institute for Cancer Research, Rye, New York

Phase I-II Trial of VM-26 (NSC-122819) in the Treatment of Children With Late-Stage Leukemia 265
Jeffrey G. Rosenstock and Milton H. Donaldson
The Children's Hospital of Philadelphia, Pennsylvania

Preliminary Report on the Use of CCNU (NSC-79037), Adriamycin (NSC-123127), and Hexamethylmelamine (NSC-13875) in Carcinoma of the Lung 269
W. L. Wilson, N. C. Andrews, R. W. Frelick, T. F. Nealon, R. L. Bick, and T. Adams
Central Oncology Group

Response of Metastatic Malignant Carcinoid Tumor to Adriamycin (NSC-123127) 273
Alan Solomon, Takuo Sonoda, and Frances K. Patterson
University of Tennessee Memorial Research Center and Hospital, Knoxville

Page

Study of Leo-1031 (NSC-134087) in Lymphocytic Lymphoma and Chronic Lymphocytic Leukemia 277
J. H. Kaufman, G. L. Hanjura, A. Mittelman, C. W. Aungst, and G. P. Murphy
Roswell Park Memorial Institute, Buffalo

Phase I Study of Weekly Administration of Anhydro-ara-5-fluorocytidine (NSC-166641) 281
P. Alberto, R. Medenica, G. Germano, and W. Bollag
University Hospital, Geneva, Switzerland

Phase II Study of Methyl-CCNU (NSC-95441) in Advanced Gastrointestinal Cancer 285
P. F. Engstrom, R. B. Catalano, and R. H. Creech
American Oncologic Hospital, Fox Chase Cancer Center, Philadelphia, Pennsylvania

A Pharmacokinetically Based Phase I-II Study of Single-Dose Actinomycin D (NSC-3053) 289
Robert S. Benjamin, Stephen W. Hall, Michael A. Burgess, Warren L. Wheeler, William K. Murphy, George R. Blumenschein, and Jeffrey A. Gottlieb
The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston

Neurotoxicity From 5-Fluorouracil (NSC-19893) Administration Reproduced by Mitomycin C (NSC-26980) 293
Michael Nichols, Patrick R. Bergevin, Avinash C. Vyas, and Robert Hamlin
Coney Island Hospital, Brooklyn, New York

Staging Colorectal Cancer: Is There a Role for Liver Biopsy? 295
Barry Rosenbloom, Jerome B. Block, and Yosef Pilch
University of California at Los Angeles-Harbor General Hospital, Torrance

ANNOUNCEMENT

Symposium on "Progress, Promise and Controversy in Cancer Chemotherapy" 297

The Secretary of Health, Education, and Welfare has determined that the publication of this periodical is necessary in the transaction of the public business required by law of this Department. Use of funds for printing this periodical has been approved by the Director of the Office of Management and Budget through November 30, 1976.

LETTERS

Results of Butocin (NSC-172755) Testing Against Tumors in Animals[1]

Butocin, an antitumor thiopurine, has been reported to inhibit the growth of solid Sarcoma 180 and ascites Yoshida sarcoma in mice (1). In subsequent clinical studies, Černý (2) has reported activity against chronic myelocytic leukemia blastic crisis, and Andrysek et al (3) have reported objective responses in > 50% of patients with generalized breast carcinoma, some of whom were receiving additional chemotherapy. On the basis of these clinical results, butocin was selected for further preclinical studies by the Division of Cancer Treatment (DCT) of the National Cancer Institute. In rodent tumor screens conducted under DCT-sponsored contracts, ip butocin was ineffective against ip L1210 and P388 leukemias, ip or sc B16 melanoma, sc Lewis lung carcinoma, and im Walker 256 carcinoma, as well as sc $CD8F_1$ and C3H mammary carcinomas. Orally, butocin was inactive against L1210 and Lewis lung tumors. L1210 and Walker 256 have been reproducibly sensitive to 6-mercaptopurine (6-MP).[2] We believed it was important to search for an animal tumor which was sensitive to both butocin and 6-MP in order to judge the extent of similarity in their actions and the possible superiority of butocin.

In collaboration with Mr. I. Wodinsky at Arthur D. Little, Inc, Boston, Mass, we examined butocin in rats with ascites Dunning leukemia, a tumor known to be exquisitely sensitive to 6-MP. Butocin, as does 6-MP, elicited a high percentage of long-term survivors. Butocin was then tested against a Dunning leukemia variant developed for resistance to 2-aminopurine-6,8-dithiol (NSC-29189) and which had been shown to be cross-resistant to 14 thiopurines including 6-MP and 6-thioguanine. Butocin was completely inactive against the resistant variant ascites tumor over a wide range of ip doses (50-800 mg/kg/day × 5); in the same experiment however, 100% of the rats with the parent tumor that received the optimal dose of 400 mg/kg/day × 5 survived for > 45 days.

6-MP has been reported to be moderately effective against human chronic myelocytic leukemia (although not apparently a treatment of choice) and minimally effective against human breast carcinoma (4). In view of our inability to demonstrate, using rodent tumor models, that butocin acts differently from or is superior to 6-MP, responses from clinicians comparing the reported clinical effects of butocin with the clinical effects of 6-MP would be welcome.

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November 21, 1975

Myelotoxicity and Orthostatic Hypotension of Cyclocytidine (NSC-145668)[1]

Lokich et al (1) have recommended an iv dose of cyclocytidine of 600 mg/m²/day × 10 for children. They felt that myelosuppression was tolerable at this dose level and that postural hypotension was not a problem for these patients. Because of this latter problem however, they have not recommended any particular dose for adults.

At the M. D. Anderson Hospital and Tumor Institute, Houston, Texas, our initial phase I evaluation of cyclocytidine in adults (2) showed that iv, im, and sc treatments gave similar plasma levels. Because of its ease of administration (especially in

[1]Butocin: CAS reg. No. 22181-94-8; glycine, *N*-[1-oxo-5-(1*H*-purin-6-ylthio)pentyl]-, ethyl ester. Synthesized at the Research Institute of Pharmacy and Biochemistry, Prague, Czechoslovakia.

[2]6-Mercaptopurine: NSC-755; CAS reg. No. 6112-76-1; purine-6-thiol, monohydrate.

[1]Cyclocytidine: CAS reg. No. 10212-25-6; 6*H*-furo[2',3':4,5]oxazolo-[3,2-a]pyrimidine-2-methanol, 2,3,3a,9a-tetrahydro-3-hydroxy-6-imino-, monohydrochloride.

TABLE 1.—Myelosuppression produced by sc cyclocytidine in 37 adults*†

Myelosuppression	No. of courses at dose level (mg/m²/day × 10) of— 200	240	300
Wbc count (cells/mm³)			
≥ 4000	1	2	8
3000-3999	0	2	2
2000-2999	1	4	5
1000-1999	1	2	3
< 1000	3	2	4
Platelet count (platelets/mm³)			
≥ 100,000	1	3	9
75,000-99,999	0	1	2
50,000-74,999	1	4	2
25,000-49,999	1	2	4
< 25,000	3	2	4

*Data from the phase I studies done by M.D. Anderson Hospital and a phase II study by the Southwest Oncology Group.

†Of these 37 patients only 9 (24%) episodes of postural hypotension were observed; postural hypotension was similar at all dose levels and was severe in only 1 patient.

patients with poor veins), we chose the sc route, selecting a dose of 240 mg/m²/day × 10 for patients with adequate marrow reserve and 200 mg/m²/day × 10 for patients with inadequate marrow reserve (this includes all patients who have previously had a nitrosourea) for phase II evaluation in the Southwest Oncology Group. Our experience indicated that at this dosage level both myelosuppression and postural hypotension were quite tolerable.

In our joint observations, which now include both M. D. Anderson Hospital and Southwest Oncology Group data, we noted that life-threatening myelosuppression (neutrophils < 1000 cells/mm³ and platelets < 20,000 platelets/mm³) has occurred with doses as low as 200 mg/m²/day given sc for 10 days (table 1). We have noted, as have Lokich et al (1), that myelosuppression was much more pronounced if patients had previously received a nitrosourea compound, even if 6 weeks had elapsed since its administration. In these patients, mild myelosuppression has been noted after only four doses of cyclocytidine. Even when cyclocytidine was discontinued at this point, patients still occasionally experienced life-threatening myelotoxic reactions. Because of these observations we recommend that patients should have blood cell counts taken before their fifth and eighth daily doses, and if mild myelosuppression has already developed, further doses of the drug should be withheld.

Recently, we have noted that the systemic toxic effects (nausea, vomiting, and postural hypotension) of cyclocytidine may be reduced by administering the drug in divided doses during the day. There are also laboratory data, in dogs, suggesting that desmethylimipramine may alter the development of postural hypotension (3). This is now under investigation.

Further phase II evaluation of this drug in adults can proceed using these lower dose recommendations with careful observation for myelosuppression. In light of our fairly extensive experience in adults that have demonstrated severe myelosuppression at dose levels considerably < 600 mg/m²/day × 10, and the limited experience of Lokich et al (1) (five courses in three patients) at that same dose level which they recommend as a starting dose in pediatric phase II studies, it might be wise for pediatricians to carefully monitor blood cell counts when using Lokich's recommended regimen.

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Levamisole (NSC-177023) in Clinical Immunotherapy[1]

In his recent Commentary (1), Dr. Bluming lists levamisole as a nonspecific active immunostimulant together with bacillus Calmette-Guérin, *Corynebacterium parvum,* and polyribonucleosides. This, however, is an over-simplification and may cause some confusion because levamisole, in contrast to the so-called immunostimulants, does not stimulate immunity above the normal level in man (2) or prevent the primary growth of most experimental tumors in immunologically normal animals (3-6). Levamisole acts as an antianergic chemotherapeutic agent as it restores cell-mediated immunity in immunodepressed patients (3,7-10) and prolongs the remission period. It even increases the number of long-term survivors if used as an adjunct to cytoreductive therapy in several animal cancer models (3,11-14). Available clinical data tend to confirm the usefulness of levamisole in prolonging the remission of cancer, at least after surgery for bronchial carcinoma (15,16) and after irradiation therapy for breast cancer (17). Further clinical trials with levamisole are therefore mandatory.

A further point raised by Dr. Bluming was the adequate dosing of bacillus Calmette-Guérin. Apparently, this also applies to levamisole since in the surgical-adjuvant study in lung cancer the drug does not differ from placebo in patients receiving low doses while a dramatic difference in favor of the drug is seen in patients receiving high doses (16). Presently, levamisole seems a very likely candidate for use as an adjunct to classic anticancer treatment.

[1]Levamisole: CAS reg. No. 16595-80-5; imidazo[2,1-b]thiazole, 2,3,5,6-tetrahydro-6-phenyl-, monohydrochloride.

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January 13, 1976

Selection of Carriers for Alkylating Moieties to Increase Their Antitumor Specificity

Several papers recently published in *Cancer Treatment Reports* have made suggestions for the rational design of antineoplastic agents. These suggestions have included ways to circumvent drug resistance (1), to reduce toxicity (2), and to

utilize the optimal log P to manipulate chemical structures in order to obtain some specific biologic activities (3). The carrier moiety of an alkylating agent affects the drug's spectrum and magnitude of antineoplastic activity; indeed, the physicochemical properties of the carrier function may account for the superiority of some alkylating agents over others in specific types of tumors (eg, cyclophosphamide in Burkitt's lymphoma, nitrosoureas in brain tumors). Therefore, it may be possible to design alkylating agents with carriers for the bis-chloroethyl group or another alkylating moiety which would lend specificity to the drug for tumors of certain types or locations (4). We suggest three such carriers.

The melanizing agents trioxsalen and methoxsalen localize in the skin, concentrating in functional melanocytes. Bis-chloroethyl derivatives of these agents (fig 1) may therefore have potential usefulness in the treatment of malignant melanoma.

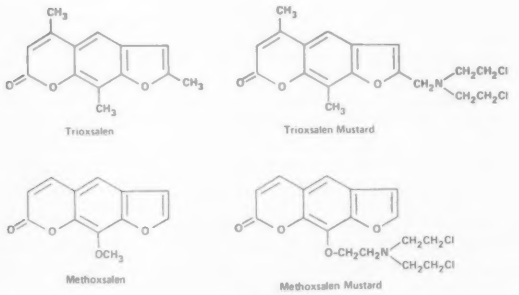

FIGURE 1

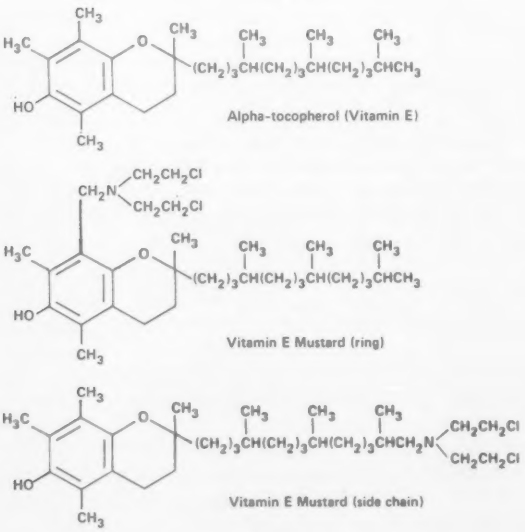

FIGURE 2

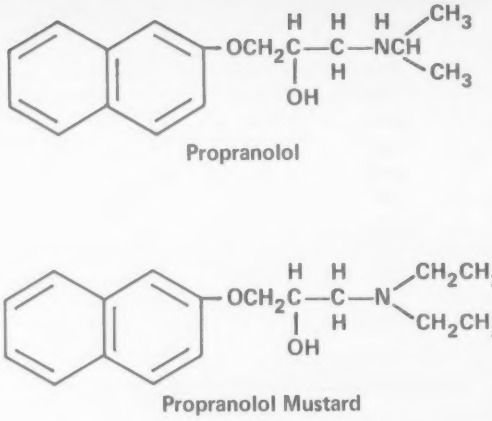

FIGURE 3

Unlike many compounds of low molecular weight (5), vitamin E is selectively absorbed by the intestinal lymphatics after oral administration (6). It is incorporated into chylomicrons that are retained within lymphatic channels until they enter the general circulation via the thoracic duct. Bis-chloroethyl analogs of vitamin E[1] (fig 2) may therefore be useful in types of malignancies which tend to metastasize through lymph channels, and particularly after the surgical removal of neoplastic tissue when tumor cells are thought to metastasize via lymphatics. Alkylating analogs of vitamin E (or vitamin E acetate, which is more stable and is not oxidized by air and light) might also be useful in the treatment of lymphomas since many tumor cells are discharged into lymphatic channels after chemotherapy or radiotherapy for this tumor.

It has been demonstrated that high levels of *dl*-propranolol accumulate in lung tissue (7) and that *d*-propranolol is also concentrated in the lungs;[2] although the *l*-isomer is an adrenergic β-blocking agent, the *d*-isomer is devoid of this activity. Bis-chloroethyl derivatives of *d*-propranolol (fig 3) may also concentrate in the lung and therefore could be useful in treating lung carcinoma.

These proposed alkylating agents should be synthesized as radioactively labeled compounds to facilitate studies of their disposition and tissue localization. Moreover, if the disposition of the bis-chloroethyl derivatives of these compounds is markedly altered, then the attachment of alternative alkylating moieties might be considered. Be-

[1]One such analog, NSC-21537, has already been synthesized; it was found to be inactive in the Drug Research and Development Program screening program but its absorption by lymphatics was not evaluated.

[2]Davies DS. Personal communication. Royal Postgraduate Medical School, London, England.

fore testing these compounds for activity against standard animal tumor systems, physiologic disposition and tissue localization studies should be performed, since the latter represents the rationale for the design of these new compounds.

We suggest that alkylating agents be designed utilizing carriers that are most likely to deliver the agents to their specific target sites. By designing drugs with activity against specific types of tumors rather than with generalized cytotoxic actions it should be possible to increase the therapeutic index of these agents.

LIST OF COMPOUNDS

Methoxsalen: NSC-45923; CAS reg. No. 298-81-7; 5-benzofuranacrylic acid, 6-hydroxy-7-methoxy-, δ-lactone
Methoxsalen mustard
NSC-21537: 6-chromanol, 5-([bis(2-chloroethyl)amino]methyl)-2,7,8-trimethyl-2-(4,8,12-trimethyltridecyl)-; γ-tocopherol, 5-([bis(2-chloroethyl)amino]methyl)-
Propranolol hydrochloride: NSC-91523; CAS reg. No. 318-98-9; 2-propanol, 1-(isopropylamino)-3-(1-naphthyloxy)-, hydrochloride
Propranolol mustard
α-DL-Tocopherol: NSC-82623; CAS reg. No. 18920-63-3; 6-chromanol, 2,5,8-trimethyl-DL-2-(4,8,12-trimethyltridecyl)-; vitamin E
Trioxsalen: NSC-71047; CAS reg. No. 3902-71-4; 5-benzofuranacrylic acid, 6-hydroxy-β,2,7-trimethyl-, δ-lactone
Trioxsalen mustard
Vitamin E mustard (ring and side-chain forms)

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DTIC (NSC-45388) Toxicity[1]

It is convenient, tidy, and perhaps safer to use the same daily dose of a drug for an entire treatment cycle. Errors in dosage are presumably less. On the other hand, some drugs such as DTIC cause relatively more nausea and vomiting during the first days of treatment despite pretreatment with prochlorperazine. After a few days the patient seems to "tolerate" the drug and the symptoms subside. Indeed, we have had two patients resume a pattern of nausea and vomiting when the drug administration was interrupted for a 3-day period.

It occurred to us that a sliding dosage scale might lessen toxicity and provide the same amount of drug in the same number of days. For example, a patient to be given 200 mg/day for 10 days may have less severe nausea and vomiting if the drug was given as follows:

Day	*Dose (mg)*
1	50
2	100
3	100
4	150
5	200
6	250
7	250
8	300
9	300
10	300

The scheduled dosage for a Monday morning may be reduced and the extra drug given on Day 10 or 11.

Avoiding or minimizing objectionable reactions is particularly desirable in patients who require multiple therapy cycles and certainly in those who are receiving prophylactic chemotherapy.

Our experience with a "shaped" course of DTIC has been good but limited. Others may wish to test this thesis.

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[1]DTIC: CAS reg. No. 4342-03-4; imidazole-4-carboxamide, 5-(3,3-dimethyl-1-triazeno)-.

Calusterone (NSC-88536) in Advanced Breast Cancer[1]

In a recent letter to *Cancer Chemotherapy Reports,* Rosso et al (1) concluded from their results with calusterone in advanced breast cancer that their data disagreed with the results published by us in this journal (2). However, the two studies are not comparable because Rosso et al used calusterone as a single agent while our results were with calusterone combined with cyclophosphamide[2] compared to fluoxymesterone[3] combined with cyclophosphamide.

It would be more valid for Rosso and co-workers to compare their results to those published by the Cooperative Breast Cancer Group (3) or with the trial published by Lemkin and Dollinger (4). In both of these trials calusterone was used as a single agent. Goldenberg et al (3), in a trial comparing calusterone to testolactone, observed clinical abnormalities, including hepatic dysfunction and gastrointestinal symptoms in 30 of 109 women treated with calusterone and in only 19 of 115 receiving testolactone. Lemkin and Dollinger (4) reported that ten of 20 women with advanced breast cancer treated with calusterone had significant symptoms attributable to drug toxicity, requiring cessation of therapy in four. Six of their patients developed pulmonary or peripheral edema, four developed significant liver function abnormalities not related to metastatic cancer, and two became hypercalcemic. They concluded that "calusterone was no more effective than conventional androgens, and was associated with an unacceptably high incidence of drug toxicity."

Of the 31 patients treated with calusterone as a single agent Rosso et al only evaluated 27. In their evaluation they excluded four patients in whom treatment was discontinued due to severe gastrointestinal side effects. In judging the acceptability or toxicity of an agent, discontinuance of a drug due to toxicity should surely be borne in mind. Rosso et al reported only a "moderate degree" of toxicity; they did, however, observe a significant rise of sulfobromophthalein levels in one third of their patients. In our randomized trial we compared cyclophosphamide plus fluoxymesterone to cyclophosphamide plus calusterone in the treatment of women with advanced breast cancer. Not only was there a better response rate with the combination using fluoxymesterone, but, of particular importance, treatment with calusterone was associated with an unacceptably high incidence of drug toxicity. Furthermore, the tolerance of patients with advanced breast cancer to cyclophosphamide was considerably better when fluoxymesterone was used compared to calusterone. The hepatotoxicity of calusterone (as indicated by elevated sulfobromophthalein levels) could explain why cyclophosphamide (an agent metabolized in the liver) is less well tolerated in combination with calusterone.

In our trial we found that calusterone was associated with considerably more side effects than a standard androgen. We could ascribe no changes in liver function to fluoxymesterone, whereas a high incidence of changes in liver function occurred in the patients receiving calusterone. Liver changes were reversed when calusterone was discontinued. Neurologic changes, hypercalcemia, and fluid retention were more often seen in patients receiving calusterone compared to those receiving fluoxymesterone, and these side effects were much more severe in calusterone-treated patients. Pulmonary edema was not seen in patients receiving fluoxymesterone but occurred in five of the 28 patients receiving calusterone. This toxic effect was considered to be a possible contributing cause of death, and in two patients this suspicion was confirmed at postmortem examination. Lemkin and Dollinger reported pulmonary or peripheral edema in six of their 20 patients.

Rosso et al reported a response rate of 29.2% for calusterone. Lemkin and Dollinger found a response rate of 20%, and in the 109 patients treated by the Cooperative Breast Cancer Group a response rate of 28% was recorded. Since it has now been conclusively shown that response rates of approximately 60% or more can be obtained when advanced breast cancer is treated with combination chemotherapy (5-7), there is no justification for the treatment of this disease with a single agent which produces remissions in < 30% of patients.

In view of the "unacceptably high incidence of drug toxicity" encountered by workers in the United States who used calusterone as a single agent, and also by us when calusterone was used in combination with cyclophosphamide, we feel that calusterone is a poor substitute for conventional androgens and that there is no place for this agent in the management of advanced breast cancer.

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[1]Calusterone: CAS reg. No. 17021-26-0; testosterone, 7β,17-dimethyl-.

[2]Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate.

[3]Fluoxymesterone: NSC-12165; CAS reg. No. 76-43-7; androst-4-en-3-one, 9-fluoro-11 β,17β-dihydroxy-17-methyl-.

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Prolonged Survival of Patients With Extensive Oat Cell Carcinoma Treated With Radiotherapy and Cyclophosphamide (NSC-26271), Vincristine (NSC-67574), and Methotrexate (NSC-740)[1]

During the past 3 years, combination therapy for oat cell carcinoma of the lung has improved the dismal natural history of this disease, for which the median survival had been 3 months (1). Bergsagel et al (2), who treated this disease by using radiotherapy and intermittent high doses of cyclophosphamide for localized tumor, found an improvement in the median survival compared to that of patients receiving radiotherapy alone. Tucker et al (3) obtained similar results by using cyclophosphamide in combination with radiotherapy. Recently, Eagan et al (4) reported a median survival of 9.5 months in patients with oat cell carcinoma treated with radiotherapy and combination chemotherapy. Similar survivals have been reported by Holoye and Samuels (5) and by Nixon et al (6). In our study, we used a protocol similar to that of Eagan et al (4) and confirmed his early results.

Eighteen patients with oat cell carcinoma of the lung were referred to the University of Chicago Hospitals and Michael Reese Medical Center during the study interval of March 1, 1974, to August 30, 1975. The diagnosis of oat cell carcinoma was made by bronchoscopy in nine patients, mediastinoscopy in two, open or closed lung biopsy in four, lymph node biopsy in two, and liver biopsy in one. Liver, bone, and brain scans were done on all patients for assessment of the clinical stage. Eight patients also had gallium-67 scans and 14 had Jamshidi needle biopsies of the posterior iliac crest. Seventeen of 18 patients were in stage III (eight with bone involvement, eight with liver metastases, and one with extrathoracic lymph node involvement) and one patient was in stage II. Four of the 14 Jamshidi needle biopsies were positive (28%), as were seven of the eight gallium-67 scans (87.5%). Monthly chest roentgenograms and measurements of clinical parameters were supplemented by quarterly liver, bone, and brain scans which provided objective parameters for assessment of the response. Radiotherapy, at a total dose of 3000-3500 rads, was administered to the primary tumor, mediastinum, and supraclavicular lymph nodes of 14 patients prior to the start of chemotherapy or between the second and third cycles on Days 56-70. Two patients received radiotherapy at dose levels > 3500 rads at other institutions. Two patients received no radiotherapy. Chemotherapy consisted of cyclophosphamide, vincristine, and methotrexate (CVM): cyclophosphamide, 2.0 g/m^2 given on Day 1; vincristine, 1.5 mg/m^2 given on Day 1; and methotrexate, 30 mg/m^2 given on Day 22. Five patients received a modified methotrexate dose of 120 mg/m^2 on Day 21, followed 24 hours later by citrovorum factor, 100 mg/m^2 in four divided doses.[2] Cycles of chemotherapy were given every 28 days, with the following modification for cyclophosphamide: 75% of the dose was given if the white blood cell (wbc) count was < 5000 but > 4000 cells/mm^3 or if the platelet count was >100,000 cells/mm^3; 50% was given if the wbc count was < 3000 but > 2000 cells/mm^3 or if the platelet count was 50,000-74,000 cells/mm^3; and no therapy was given if the wbc count was < 2000 cells/mm^3 or if the platelet count was < 50,000 cells/mm^3.

Responses to combination therapy were classified as CR if there was disappearance of all meas-

[1]Cyclophosphamide: CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate.

Vincristine: CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1).

Methotrexate: CAS reg. No. 59-05-2; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-.

[2]Citrovorum factor: NSC-3590; CAS reg. No. 6055-86-5; glutamic acid, *N*-[*p*-[[(2-amino-5-formyl-5,6,7,8-tetrahydro-4-hydroxy-6-pteridinyl)methyl]amino]benzoyl]-, calcium salt.

urable clinical, radiologic, and scintigraphic evidence of disease. PR was defined as a ≥ 50% decrease of all measurable lesions (sum of the products of the greatest diameter and its perpendicular). The appearance of a new lesion or no change in the size of any measured lesion was termed no response.

Survival is shown in figure 1. There were three CRs and ten PRs, giving a total response rate of 72%. The duration ranged from 3 to 13+ months. Patients who responded usually did so within the first two cycles of chemotherapy. The median survival of the 13 responders (CR and PR) was 11.0 months versus 1.8 months for the five nonresponders; this difference is statistically significant ($P = 0.01$). Separating the group of responders into the nine patients who received the low dose of methotrexate (30 mg/m^2) and the five patients who received the higher dose (120 mg/m^2), we find no differences. One of the five patients treated with the higher dose of methotrexate failed to respond. Of the four patients who responded, one had a CR and three had a PR.

The mean cyclophosphamide dose was 82% of the calculated dose for each cycle. The mean doses of vincristine and methotrexate were nearly 100% per cycle. Severe reduction in the platelet count to < 50,000 cells/mm^3 was not observed in any of the patients. One drug-related death occurred; the patient had a PR, but died of an overwhelming bacterial pneumonia and sepsis with a wbc count of < 1000 cells/mm^3. Drug toxic effects included hemorrhagic cystitis in one patient. Nausea, vomiting, and alopecia occurred in all patients. Acral paresthesias were present in ten of the 18 patients; however, there were no cases of severe sensorimotor defects with loss of vibratory sense or muscle weakness. No instances of liver dysfunction or renal toxicity were noted in the patients who received high doses of methotrexate. Severe drug-related anemia was not observed.

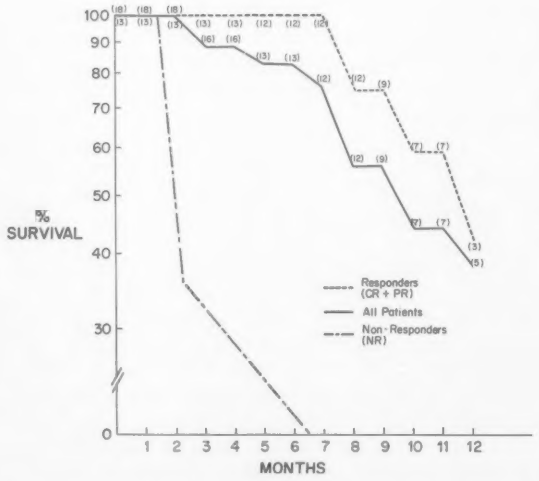

FIGURE 1.—Cumulative survival curves for all patients, including responders (CR + PR) and nonresponders.

Although radiotherapy has been able to control this dread disease, it has not improved overall survival. The use of combined radiotherapy and combination chemotherapy in patients with oat cell carcinoma has lengthened survivals to 9.5-10 months (4-6). While the survival of our 18 patients with oat cell carcinoma who were treated with CVM and radiotherapy appeared to be only 3.0 months longer than for those treated with cyclophosphamide and radiotherapy, it should be noted that all of our patients had extensive disease (stage III) and that those treated with radiotherapy and cyclophosphamide had stage II disease.

We have confirmed the results reported by Eagan et al (4) in a group of 18 patients, 16 of whom had extensive bone or liver disease. The median survival of all patients treated was 9.0 months. Responders (CR and PR) had a median survival of 11 months which was attained with negligible toxicity. There was a very significant difference in survival between responders (CR and PR) and nonresponders, 11 months versus 1.8 months respectively ($P = 0.01$). It is possible to predict which patients will respond since objective response is usually seen within the first two cycles of chemotherapy. Our studies confirm the successful use of CVM combined with radiotherapy in patients with oat cell carcinoma and extend the indications to patients with extensive disease.

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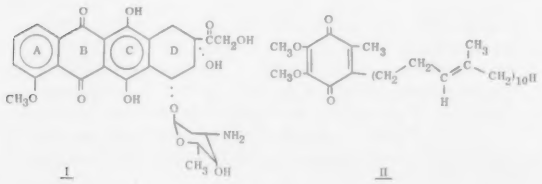
[3]Operated by the University of Chicago for the US Energy Research and Development Administration under contract E(11-1)-69.

Prevention by Coenzyme Q_{10} (NSC-140865) of the Inhibition by Adriamycin (NSC-123127) of Coenzyme Q_{10} Enzymes[1,2]

Rings B and C of adriamycin in structure I below are depicted as quinoid and hydroquinoid respectively, but these functionalities are presumably tautomeric. The relationship between this quinoid nature of adriamycin and that of coenzyme Q_{10} (CoQ_{10}) (II) is evident.



I II

[1]Supported by grants from the John A. Hartford and the Robert A. Welch Foundations.

[2]Coenzyme Q_{10}: p-benzoquinone, 2-(3,7,11,15,19,23,27,31,35,39-decamethyl-2,6,10,14,18,22,26,30,34,38-tetracontadecaenyl)-5,6-dimethoxy-3-methyl-; coenzyme Q-199.

Adriamycin: CAS reg. No. 23214-92-8.

It was anticipated that adriamycin, as well as carminomycin and daunorubicin, could inhibit the CoQ_{10} enzymes, succinoxidase and NADH-oxidase, from heart tissue. This anticipation of Iwamoto et al (1) resulted in data which demonstrated that these three anthracyclines did indeed inhibit mitochondrial preparations of CoQ_{10} enzymes from the tissue of beef heart, and of these three compounds adriamycin was the most potent inhibitor. The potency of the 14-octanoate supports the interpretation that adriamycin can indeed function in these lipoidal enzyme systems. This inhibition by adriamycin and its 14-octanoate can be explained by the quinoid nature of rings B and C and the greater lipoidal character of the ester.

The inhibition by adriamycin supports the concept (1) that the antitumor activities of adriamycin could be due, at least to a minor extent, to inhibition of CoQ_{10} enzymes in the electron-transfer processes of cell respiration in tumor tissue and that in addition this mechanism of inhibition could be based to a major extent on intercalation within DNA helices. The antitumor activities of many quinones are well known, and analogs of CoQ_{10} have been found to inhibit CoQ_{10} enzymes in vitro and to have antitumor activity in vivo (2,3), particularly 2,3-dimethoxy-5-chloro-6-octylmercapto-1,4-benzoquinone.

In 1974, subsequent to the data published in April of that year (1), it was considered that the cardiotoxicity of adriamycin, a severe side effect of the administration of this drug to cancer patients, could be due to the inhibition by adriamycin of CoQ_{10} enzymes in the mitochondria of the cardiac tissue of the patients. The well-known high level of mitochondria in cardiac tissue and the concomitant high concentration of CoQ_{10} in such mitochondria gave additional credence to the concept of the implication of damage by adriamycin of these cardiac CoQ_{10} enzymes as a significant aspect of the toxicity.

If a significant cause of the clinical cardiotoxicity of adriamycin is the inhibition by adriamycin of CoQ_{10} enzymes, then the prevention or reversal of this cardiotoxicity in a cancer patient is of clinical importance. Preliminary data on the prevention by supplementary CoQ_{10} of the inhibition by adriamycin of CoQ_{10} enzymes were reported at the Oncology-Cardiology Workshop on Adriamycin Cardiomyopathy at Wright State University on September 29, 1975. These data have since been considerably expanded and are discussed below.

The antimetabolite-CoQ_{10} index for a sample of adriamycin (mp 206°C [dec]) was 906, which signifies an inhibitory activity greater than that (1475) of a previous sample with a lower melting point (mp 156°C-176°C [dec]) (*see* ref 1). When this sample

of adriamycin was used at a concentration of 2.5 μmol/flask (2.8 ml), the specific activity (μatoms of O_2/mg/min) was 0.15 and the relative activity of succinoxidase was 30%. In comparison, the specific activity of the control was 0.499 and the relative activity was assigned at 100%. In the presence of only a fourfold molar excess, 10 μmols of supplementary CoQ_{10}, a relative activity of 104% was observed, which showed that the potential inhibition by 2.5 μmols of adriamycin had been prevented.

Next, five determinations of the inhibition by 2.5 μmols of adriamycin gave a mean relative activity of 31% ± 2% SD. In the presence of 10 μmols of supplementary CoQ_{10}, the potential inhibition by 2.5 μmols of adriamycin was prevented and the relative activity was 116% ± 7% (five determinations) ($P < 0.001$).

In 1974 Gosalvez et al (4) reported that adriamycin and daunorubicin inhibited mitochondrial respiration by nearly 100% at a 1 mM concentration, and that it is state 3 but not state 4 respiration which is inhibited.

Our data on the inhibition by or toxicity of adriamycin to mitochondrial CoQ_{10} enzymes and its prevention by CoQ_{10} are in agreement, in principle, with results reported in June-August of 1975 by Bertazzoli et al at Farmitalia, Milan, Italy. Bertazzoli et al (5) described the antagonistic action of ubiquinone (CoQ_{10}) on the experimental cardiotoxicity of adriamycin in the isolated rabbit heart. Bertazzoli et al (6) next reported that the cardiotoxicity of adriamycin in vivo in rabbits was prevented by ubiquinone (CoQ_{10}).

Adamson (7) proposed a hypothesis in 1974 that the cardiac toxicity of daunorubicin and adriamycin could be due to the amino sugar moiety of the molecules. In 1975 Wheeler (8) proposed a related hypothesis concerning the cardiotoxicity, and suggested that 6-deoxy derivatives "should be less damaging to the heart," based on intercalation and hydrogen bonding. Besides Wheeler's rationale, the 6-deoxy derivatives could partially limit the quinoid functionality toward reducing inhibition of CoQ_{10} enzymes.

It is our current view that the antitumor activity of adriamycin can be largely due to mechanisms of intercalation within DNA helices and, to a minor extent, to inhibition of CoQ_{10} enzymes in tumor tissue (9). The cardiotoxicity of adriamycin can be largely due to inhibition of CoQ_{10} enzymes which are present in cardiac tissue to a high extent, because of the high level of mitochondria supporting bioenergetics. Since CoQ_{10} exists naturally in the human myocardium and is indispensable for the electron-transfer processes of respiration and coupled phosphorylation, it is evident that significant inhibition of cardiac CoQ_{10} could be highly detrimental to cardiac function and the welfare of the cancer patient.

Since this cardiotoxicity is a most serious side effect in cancer patients and limits the therapy with adriamycin, it is now timely for clinical studies of the combination of CoQ_{10} and adriamycin in cancer. The administration of CoQ_{10} could be before, during, or after adriamycin. Since CoQ_{10} enzymes are presumably in all cells of the human body having mitochondria, it is quite possible that other major side effects of adriamycin, including hematologic toxicity, could be minimized or prevented by such concomitant treatment with CoQ_{10}.

The separation of antitumor activity based on intercalation with DNA from clinical side effects based on inhibition of CoQ_{10} enzymes can be a specific rationale for the syntheses of new less toxic analogs of adriamycin for cancer therapy.

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March 1, 1976

Laser Differential Light-Scattering Bioassay for Methotrexate (NSC-740)[1,2,3]

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SUMMARY

A new bioassay method utilizing laser light scattering from suspensions of drug-sensitive bacteria has been developed for the estimation of antitumor drugs in biologic samples. Changes in the light-scattering patterns of antibiotic-treated bacteria have recently been shown to provide a rapid and accurate indication of antibiotic sensitivity. Similar considerations for several antitumor drugs have shown the method capable of assaying 0.1 ml with drug concentrations as low as a few nanograms of drug per milliliter of sample. The first successful application of the methodology is described for the antitumor agent methotrexate. Studies of both drug-treated human serum specimens and dog serum levels and urinary excretion as a function of time indicate that assay results are available within 3 hours of preparing the serum. Time variations of drug serum levels and urinary excretion rates are compared via laser differential light-scattering assay, standard disc-diffusion assay, and previously published radioisotopic assays.

[Cancer Treat Rep 60:225-233, 1976]

The response of bacteria to antitumor agents is often similar to their response to antibiotics (1). Broad variations in susceptibility patterns to antitumor agents are found among the many species and strains isolated and used for assay purposes (2). Such assays are conventionally performed by applying small aliquots of the test solution to filter paper discs placed on agar-nutrient substrates (2) previously seeded with a suitably chosen test organism. After incubation, the zones of inhibition surrounding the discs are measured and interpreted via a simultaneously determined zone-versus-known concentration standard curve. Such microbial assay techniques are particularly useful for certain drugs because of the relative ease by which the test may be set up and performed. In addition, such assays obviate the need for the administration of radioisotopes, are quite sensitive, and require only small specimens. The main shortcomings of such assay procedures relate to their inherent slowness, their requirement for technicians skilled in the reading and interpretation of inhibition zone diameters, and their critical dependence on inoculum size and media composition.

During the past few years a new bioassay technique for antibiotic susceptibility testing has been developed (3,4) that is based upon measurements of certain light-scattering properties of treated bacteria, ie, their *differential light-scattering* (DLS) patterns. Such patterns correspond to a recorded measurement of the intensity of light scattered from a bacterial suspension as a function of detector angle. Figure 1 illustrates this type of measurement with the direction of the incident light beam taken as the reference direction, $\theta = 0°$. A suspension of bacterial cells ($\sim 10^6$/ml) contained in a conical cuvette[7] is placed in a fine laser

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[2]Supported by contract N01-CM-43780 from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare.

[3]Methotrexate: CAS reg. No. 59-05-2; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-.

[4]Science Spectrum, Inc, Santa Barbara, Calif.

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[6]The assistance of Dr. Salah El Dareer, Susan Wilson, Beverly Washburn, Vivian White, and Stephen Chappell is gratefully acknowledged. We also acknowledge the assistance of Dr. Robert D. Hill in planning some of the experimental strategies and helping in the error analysis.

[*]*Reprint requests to:* Dr. L. B. Mellett, USV Pharmaceutical Corp, 1 Scarsdale Rd, Tuckahoe, NY 10707.

[7]Schoefer P, and Berkman RM. Sample cell. US patent No. 3,701,620.

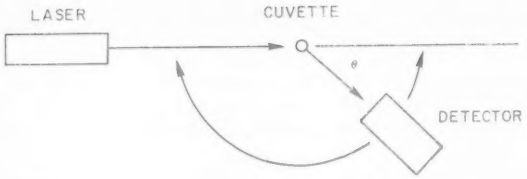

FIGURE 1.—Schematic diagram of a DLS measurement. The laser source illuminates a suspension of cells in a cuvette. The detector measures the scattered light intensity at angle θ as it rotates about the cuvette. The acceptance angle of the detector is 2°.

beam which passes diametrically through the cuvette. Typically, the cuvette diameter is about 2.5 cm and the laser beam diameter is about 2 mm, thereby illuminating a volume containing about 10^5 bacteria (3). A collimated detector with an acceptance angle of 2° receives the scattered light only from the illuminated cells near the center of the cuvette and generates a signal proportional to the intensity of the light detected. About 5000 cells contribute to this signal at any angular position of the detector. The DLS pattern previously referred to is a recording of the scattered light intensity variation as a function of the angle (detector position).

The calculation of a response (represented as a score) related to the drug concentration present in the specimen is derived by a comparison of the measured DLS pattern with that of a reference control (3).

Figure 2 contrasts three DLS patterns. The lowest curve was obtained from a cuvette containing 15 ml of demineralized water, and the upper two curves were obtained from two different suspensions of uniform polystyrene latex spheres.

These latex suspensions were made by combining 0.3 and 0.1 ml of a polystyrene latex stock solution (Dow Chemical polystyrene LS-166B) with 14.7 and 14.9 ml respectively of demineralized water to yield total volumes of 15 ml.

It should be noted in figure 2 that the features of the upper DLS pattern between 50° and 60° appear somewhat smoothed out with respect to the middle DLS pattern (made from 0.1 ml latex). This change is due to multiple scattering effects and is unrelated to changes in particle size or morphology. It represents a simple means by which such morphologic changes may be simulated for control purposes (3).

The average diameter of a suspended particle is 1.2 nm. With time, such reference solutions deterio-

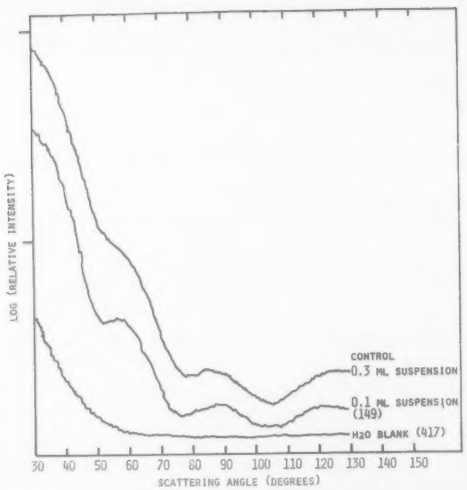
LOG (RELATIVE INTENSITY)
CONTROL
0.3 ML SUSPENSION
0.1 ML SUSPENSION (149)
H2O BLANK (417)
30 40 50 60 70 80 90 100 110 120 130 140 150
SCATTERING ANGLE (DEGREES)

FIGURE 2.—DLS patterns and scores of standard polystyrene-latex suspensions and demineralized water. (Scores in parentheses are relative to the control.)

rate by forming agglomerates which in turn cause an attenuation of the features of the associated DLS pattern.

The DLS patterns, such as those shown in figure 2, are sensitive functions of the number, average size, shape, structure, and size distribution of the scattering particles. Conditions that can affect any or all of these parameters will result in correspondingly changed DLS patterns. Extensive studies (3,5) have shown that most bacterial strains yield changed DLS patterns (relative to a control) after a 1-2-hour exposure to the antibiotics to which they are susceptible. Furthermore, the degree of change is dependent on the antibiotic concentration itself (3). For some strains, the variation of DLS pattern-change with antibiotic concentration is sufficiently great and reproducible so that the method is immediately applicable for rapid quantitative assay. Accordingly, it has been the objective of the present study to determine the potential application of a similar bioassay technique for antitumor drugs.

The use of DLS techniques for assay purposes requires a quantitative comparison of DLS patterns from control and treated suspensions. Although such comparisons may be done directly (3) from the recorded patterns, the numerical procedures are tedious and time consuming. A simpler and far more rapid approach consists of storing the patterns digitally and then comparing them in real time by means of a small digital computer. As

this has already been achieved in the DIFFERENTIAL III[8] architecture, we have made direct use of this system for our assays.

The DIFFERENTIAL III was developed specifically as an antibiotic susceptibility testing device (4,6). As such, it produces a punched card containing "scores" from 0 to 1000 for up to nine different antibiotics. These scores are a measure of the bacterial response to each corresponding antibiotic and are used to judge the relative drug effectiveness. The DIFFERENTIAL III generates scores on the basis of a stored algorithm. The algorithm compares the changes of the DLS patterns due to: (*a*) changes in cellular morphology which cause changes in the shape of the DLS pattern; and (*b*) changes in the cell density which cause vertical displacement of the DLS pattern. Such changes, and the scores generated directly therefrom, are sensitive functions of the incubation conditions and times, drug mode of action, bacterial strain, etc (3). To use this instrumentation as an assay tool requires, therefore, that a standard protocol be established for each drug to be tested. A standard response curve is then obtained which presents the score versus concentration. Unknown specimens are then assayed with respect to this standard much in the same manner as a more traditional bacterial bioassay would be interpreted.

We are reporting our first application of DLS techniques as an assay procedure for the estimation of the antimetabolite methotrexate. Although the protocols described have given us excellent results, we should point out that they are by no means final or optimal. For example, for our choice of a susceptible organism we chose the strain that has become the standard for the traditional bioassay method (2). Obviously, we could have selectively screened for an organism that might have yielded an even better DLS response. We would expect that other choices of bacteria, media, and protocol could be expected to work as well or perhaps significantly better. For the present work, however, we have selected a set of experimental procedures that nevertheless produce exceptional results.

MATERIALS AND METHODS

The suspensions whose DLS patterns are displayed in figure 2 served as the daily calibration standard for the DIFFERENTIAL III. With the 0.3-ml latex suspension in the "control" position, the 0.1-ml suspension and water blank produce scores of 149 and 417 respectively. The day-to-day variation in these scores is 5% and any major departure from these values is an indication of severe sample deterioration or system failure. New samples are prepared as needed (generally on a weekly basis) to avoid the first possibility.

The bacterial inoculum was prepared starting with an overnight (18-hour) culture of *Streptococcus faecalis* ATCC-8043. The overnight culture was grown in folic acid assay medium broth culture (FAAM, Difco) supplemented with 0.001 μg of folic acid per milliliter. An aliquot of this broth culture was then added to prewarmed (39°C $\pm$ 0.5°C) FAAM broth supplemented with 0.00133 μg of folic acid per milliliter until a transmittance of 80% was obtained with a Bausch and Lomb Spectronic 20 spectrophotometer (λ = 660 nm). After 30 minutes' incubation this new culture became the standard inoculum.

A 0.3-ml volume of the standard inoculum was carefully added to and combined with a 0.1-ml aliquot of the fluid to be assayed at the bottom of an 18 × 150-mm test tube. The solutions were maintained at 39°C $\pm$ 0.5°C in a waterbath for 1 hour and then diluted with 12 ml of 39°C solvent: one part FAAM broth supplemented with 0.002 μg of folic acid per milliliter to two parts of demineralized water. After allowing 90 minutes for equilibration in the 39°C waterbath, the cultures were chilled in 2°C ice water for 30 seconds and were then poured into a conical cuvette.[9] The DLS reading was then made on the DIFFERENTIAL III unit. Each specimen was prepared in triplicate and its DLS pattern was measured twice, thus producing six readings. The average score was taken as representative of the result. The results are expressed as micrograms of methotrexate equivalents per sample.

Standard curves (scores vs log concentration) were generated immediately preceding a particular assay using filtered pooled serum or urine to which selected amounts of methotrexate had been added. Samples were diluted in control serum or urine, and unknown concentrations were then deduced by interpolation from the standard curve.

Recovery of methotrexate added to control serum and urine averaged 98% $\pm$ 9% compared to saline drug standards. All materials and solutions were filter-sterilized before use.

It should be noted that the filtration procedures required for the DLS assay are specifically designed to remove large particulate debris that could interfere with the assay itself. Bacterial contamination is not usually a problem (even for specimens that have been incubated several hrs)

[8]DIFFERENTIAL III is a registered trademark of Science Spectrum, Inc, Santa Barbara, Calif.

[9]*See* footnote 7.

since the assay time is too brief to permit the contaminant to overwhelm the assay inoculum. Accordingly, many facets of the DLS assay may be achieved with no special care required to avoid accidental minor contaminations.

Four female beagle dogs weighing between 9.4 and 11.5 kg received methotrexate at an iv dose of 1.0 mg/kg of body weight. Blood was collected before treatment and at 5, 15, and 30 minutes and 1, 2, 4, and 8 hours after treatment. All urine samples were collected by means of an indwelling catheter for the periods 0-2, 2-8, and 8-24 hours. All specimens were frozen after suitable preparation (collection of serum from blood samples). Standards were also prepared using pooled serum and urine.

Disc diffusion assays were conducted by impregnating 1.27-cm diameter filter paper discs (No. 740-E, Schleicher and Schuell Co, Keene, NH) with 0.08 ml of solutions to be assayed or appropriate dilutions of these solutions. Standards were also run at various concentrations. The discs were placed on the surface of solidified FAAM agar supplemented with 0.002 μg of folic acid per milliliter and seeded with *S. faecalis* ATCC-8043. After an 18-hour incubation, the zones of inhibition surrounding the discs were measured. The standard curves of zone diameter versus log of drug concentration were prepared and the unknown concentrations of methotrexate equivalents were then determined. All samples and standards were run in triplicate. Details of this method have been previously published (2), though the method has now been modified in the following respect: seeded agar (pour plates) has replaced the agar overlay step, and the incubation temperature has been changed to 30°C. With these modifications the sensitivity of the procedure has been increased to about 0.008 μg/ml.

RESULTS AND DISCUSSION

Figures 3-5 show typical DLS patterns and mean scores recorded during the preparation of a standard dose-response curve. A standard curve is prepared for each assay. These figures contrast the pattern of the control specimen (no drug) with patterns of specimens treated with 1.0, 0.1, and 0.01 μg of methotrexate per milliliter respectively. Each figure shows in parentheses the corresponding score for each concentration.

Figure 6 shows a standard dose-response curve from the DLS measurements. The standard deviations of the mean values for each concentration are also indicated in the figure. The linear response range is between 1.0 and 0.003 μg/ml. Samples containing concentrations > 1.0 μg of metho-

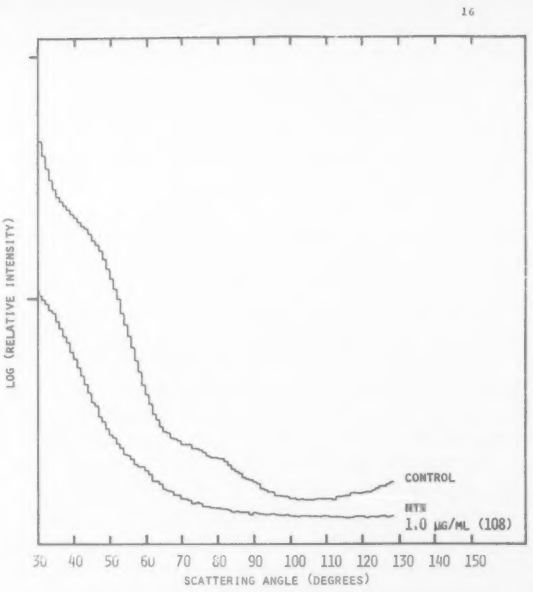

FIGURE 3.—DLS pattern and score (in parentheses) recorded for a control *S. faecalis* ATCC-8043 suspension and a similar suspension treated with 1.0 μg/ml of methotrexate (MTX).

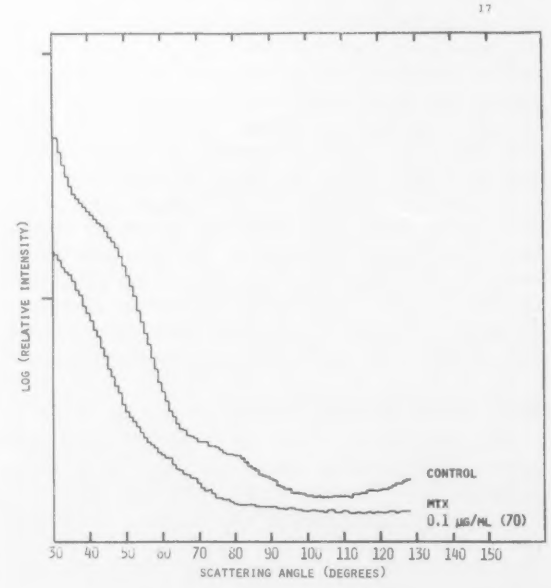

FIGURE 4.—DLS pattern and score (in parentheses) recorded for a control *S. faecalis* ATCC-8043 suspension and a similar suspension treated with 0.1 μg/ml of methotrexate (MTX).

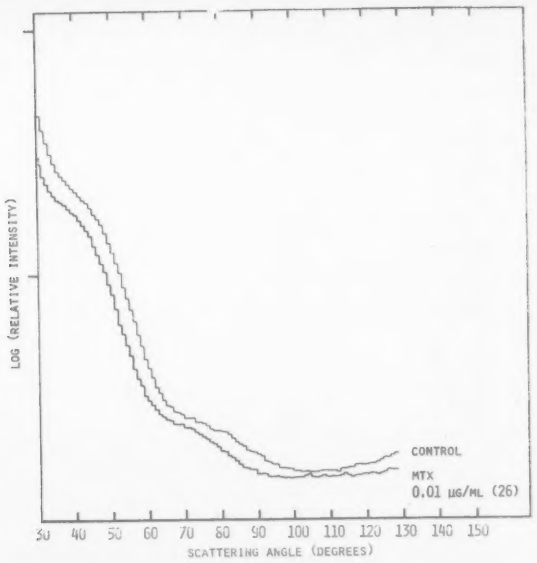

FIGURE 5.—DLS pattern and score (in parentheses) recorded for a control *S. faecalis* ATCC-8043 suspension and a similar suspension treated with 0.01 µg/ml of methotrexate (MTX).

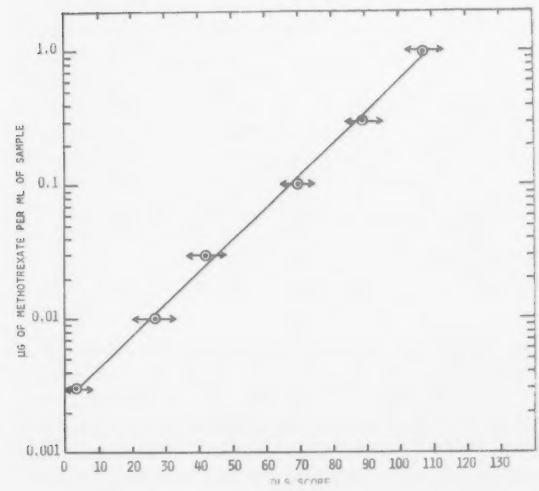

FIGURE 6.—Standard serum response curve. DLS score vs concentration of methotrexate (µg) per ml of sample.

trexate per milliliter must be diluted appropriately to provide scores within the linear range of response. A response curve for the various drug concentrations in urine was also constructed. The curves for serum and urine are similar. The error for any given point on the standard curve is approximately 10% (7).

In dealing with samples of totally unknown concentrations, we initially found it necessary to prepare multiple dilutions to ensure that certain of the determinations would fall within the linear range. This problem is also pertinent to the disc diffusion assay. However, with experience, it is possible to anticipate the expected concentration range for a given type of determination so that two or three dilutions of an original sample will provide values falling within the linear range. The dilution technique does provide an additional means of verification of results since two or more dilutions of the same samples falling within the linear score range should give equivalent values when corrected for the dilution factor.

Table 1 compares the serum bioassay results for both the disc diffusion and the DLS assay methods. The initial analysis of the serum samples from dog No. 6122 gave reasonable agreement between the two methods. However, repeated analysis by the DLS assay of the serum samples of this dog and dog No. 6044 produced declining values with each re-analysis. Between analyses, the samples were frozen and then thawed just prior to re-analysis. These observations indicated a deterioration of methotrexate in the samples.

Approximately 55 days after drug administration to the animals and after repeated freezing and thawing of the serum samples, they were re-analyzed by both the disc and DLS assays. The original standards which had also been frozen and thawed repeatedly as well as freshly prepared standards were also assayed by both methods. The results for both assays, though lower than the original values, agreed quite well. The original serum standards also showed a marked deterioration of drug compared to the freshly prepared standards. It seems apparent that the prolonged storage of samples with repeated freezing and thawing is to be avoided.

The results of serum level determinations of methotrexate in dog Nos. 1169 and 6127 for both methods are also shown in table 1. These samples were analyzed 24 hours after drug administration. The agreement between the results of both methods, with few exceptions, is excellent. For comparative purposes, serum level values of methotrexate obtained by a tritium-labeled drug assay are also shown. These data were derived from the work of Henderson et al (8) and from the data and pharmacokinetic modeling of Bischoff et al (9). Over the time course followed there is good agreement between the three methods and a somewhat closer

TABLE 1.—Serum levels of methotrexate in μg/ml ± SD in dogs receiving 1.0 mg/kg iv

	Dog No.—										
	6122		6122		6044		1159		6127		
	Assay		Assay		Assay		Assay		Assay		Tritium-labeled methotrexate assay†
Time (min)	Disc (12/10/74)*	DLS (1/7/75)	Disc (1/27/75)	DLS (1/27/75)	Disc (2/3/75)	DLS (2/3/75)	Disc (2/11/75)	DLS (2/11/75)	Disc (2/11/75)	DLS (2/11/75)	
Before treatment	0	0	0	0	0	0	0	0	0	0	0
5	4.99±0.37	5.25±0.80	2.33±0.17	2.20±0.35	3.85±0.27	3.48±0.61	5.56±0.22	5.55±1.20	5.51±0.61	3.23±1.20	6.0
15	3.33±0.56	2.32±0.46	2.19±0.37	1.59±0.32	2.46±0.42	1.85±0.45	3.25±0.37	3.10±0.57	3.44±0.79	1.78±0.56	3.1
30	1.94±0.44	1.68±0.17	1.35±0.31	1.57±0.15	1.34±0.31	0.96±0.10	2.64±0.58	2.33±0.21	1.76±0.50	1.73±0.43	2.0
60	1.79±0.39	1.05±0.12	1.09±0.24	0.60±0.08	0.68±0.15	0.84±0.10	1.90±0.75	1.60±0.16	1.65±0.15	1.60±0.40	1.13
90	—	—	—	—	—	—	—	—	—	—	0.87
120	1.30±0.23	0.29±0.04	0.64±0.11	0.42±0.06	0.54±0.09	0.39±0.05	1.44±0.53	0.73±0.20	1.15±0.05	0.64±0.11	0.66
150	—	—	—	—	—	—	—	—	—	—	0.60
180	—	—	—	—	—	—	—	—	—	—	0.58
240	0.50±0.09	0.24±0.03	0.26±0.05	0.13±0.02	0.16±0.02	0.13±0.02	0.75±0.15	0.28±0.08	0.32±0.06	0.15±0.03	0.26
480	0.08±0.02	0.04±0.01	0.06±0.01	0.03±0.01	0.03±0.01	0.03±0.01	0.09±0.02	0.07±0.01	0.06±0.01	0.04±0.01	—

*Dates refer to date of analysis.
†Data adapted *from* refs 8 and 9.

TABLE 2.—Urinary excretion of methotrexate in dogs receiving 1.0 mg/kg iv

Collection period (hrs)	Percent of total dose recovered for various collection periods ± SD in dog No.—							
	6122		6044		1169		6121	
	Assay		Assay		Assay		Assay	
	Disc (12/10/74)*	DLS (12/31/74)	Disc (12/10/74)	DLS (12/31/74)	Disc (2/11/75)	DLS (2/14/75)	Disc (2/11/75)	DLS (2/19/75)
0-2	44.6 ± 7.5	53.5 ± 9.2	45.6 ± 7.8	46.5 ± 7.9	49.5 ± 10.4	47.2 ± 13.0	27.5 ± 2.3	33.3 ± 2.4
2-8	30.7 ± 6.4	32.7 ± 4.5	41.0 ± 8.5	35.2 ± 7.2	32.9 ± 8.5	29.3 ± 4.3	13.6 ± 2.3	15.0 ± 2.3
8-24	16.8 ± 2.6	10.0 ± 1.7	2.9 ± 0.4	1.0 ± 0.2	13.0 ± 3.2	13.0 ± 3.2	18.7 ± 4.0	12.4 ± 2.8
Total	92.1 ± 5.5	96.2 ± 5.1	89.5 ± 5.6	82.7 ± 5.1	94.5 ± 7.3	89.5 ± 6.8	59.8 ± 2.8	60.7 ± 2.5

*Dates refer to date of analysis.

agreement between the DLS and isotope assays at later time periods.

Table 2 compares the results of urinary excretion studies in all four dogs obtained by the disc diffusion assay and the DLS assay. The percent of total dose recovered for each collection period and the total 24-hour recovery ± SD are shown. With minor exceptions, the agreement of the results obtained with the two methods is good. In limited studies of the urinary excretion of tritium-labeled methotrexate in dogs, Henderson et al (8) reported a 70% recovery in 24 hours. In more extensive studies in man, Henderson et al (10) reported recoveries of 58%-92% in urine in 24 hours. These results and the results presented here indicate that the dog and man excrete about the same amount of methotrexate in 24 hours. Drug decomposition noted in stored serum samples was also observed in stored and repeatedly frozen and thawed urine samples.

It should be emphasized that all unknown concentrations of drug in serum or urine specimens are determined by means of comparison with standard concentrations of methotrexate in control serum or urine. Thus, in reference to the parent drug, all unknown concentrations are expressed as micrograms of methotrexate or methotrexate equivalents per milliliter of sample.

The DLS and disc assays differ in regard to the speed and labor required to perform each. Table 3 shows the specific tasks characteristic of each assay and table 4 breaks down each task into its associated time requirements, ie, elapsed (or waiting) time and labor (or direct) time. From these tables we see that preliminary preparation time (tasks 1 and 2) for the two methods is substantially different: the DLS assay requires 21 minutes of elapsed time and 11 minutes of labor time, whereas the disc assay requires 85 and 21 minutes for elapsed and labor time respectively. The elapsed and labor times for the subsequent specimen assay tasks (tasks 3-6 for the DLS assay and 3-7 for the disc assay) are 154 and 9 minutes respectively for the DLS assay in contrast to the corresponding times of 1092 and 10 minutes for the disc assay. Total elapsed and labor time for both assays is shown in table 4.

There are some sources of inconsistency in the present technique, but all of them have now been identified and most are easily eliminated. Temperature variations have a dramatic effect on score results. Accordingly, we have made use of a waterbath to maintain specimen temperatures within a narrow range (39°C ± 0.5°C). Faulty plastic cuvettes with surface scratches or other flaws can seriously distort the recorded scattering characteristics. This problem is eliminated by careful visual inspection before use. Bacterial cultures must be in their early exponential growth phase before interacting with drug-containing specimens. This has been achieved by using a heavy overnight broth culture to standardize the working culture before use. The possibility of DIFFERENTIAL III failures, though usually detected internally by special computer functions, is carefully monitored by means of frequently prepared latex standards. Additional work is in progress concerning strain selection. Although *S. faecalis* ATCC-8043 yields reasonably good results, other less fastidious strains may produce more sensitive and rapid DLS assays.

TABLE 3.—Bioassay time elements for a typical analysis

DLS assay		Disc assay	
Task No.	Tasks*	Task No.	Tasks†
1	Preparation of bacterial culture: from overnight culture prepare standard inoculum by adjustment of inoculum transmittance.	1	Preparation of bacterial culture: centrifuge overnight culture, resuspend 3 times in saline, and adjust to 20% light transmittance.
2	Prepare filtered diluent solution.	2	Prepare agar assay media: weigh, dilute, autoclave, and cool.
3	Prepare dilutions of specimens to be analyzed in triplicate.	3	Prepare dilutions of specimens to be analyzed.
4	Combine aliquots of inoculum with assay specimens and incubate 150 min.	4	Prepare assay plates in triplicate by seeding and dispensing agar. Allow seeded agar to solidify.
5	Dilute individual samples with 12 ml of solvent. Cap and read on DIFFERENTIAL III.	5	Apply specimen aliquots to discs; apply discs to plates, cover, and incubate overnight.
6	Extract concentrations from standard curve and correct for dilution factors, if any.	6	Mark and measure zones of inhibition.
		7	Extract concentrations from standard curve and correct for dilution factors, if any.

*Several steps require some care to avoid particulate contamination; therefore, specimen filtration is required.

†All steps require care to avoid bacterial contamination; therefore, all such steps must be executed under sterile conditions.

TABLE 4.—Time elements for assay

(min)

Assay					
DLS			Disc		
Task No.*	Elapsed time	Labor time	Task No.*	Elapsed time	Labor time
1	1	1	1	40	6
2	20	10	2	45	15
3	—	5	3	—	5
4	150	1	4	10	1
5	3	1	5	1080	1
6	1	2	6	1	1
			7	1	2
Total	175	20		1177	31

*Tasks 1 and 2 are required as preliminary preparations for both assays.

The DLS assay results presented above are based on numerous experiments which have resulted in the protocol described in the section on Methods and Materials. This protocol, though by no means optimal, has resulted in a remarkably successful bioassay. The results presented are exceptionally promising and are being developed into sensitive, rapid, and inexpensive methods of broad application.

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Methyl-CCNU (NSC-95441) in Advanced Colorectal Carcinoma After Failure of 5-Fluorouracil (NSC-19893) Therapy[1,2]

B.J. Cedermark,[3] M.S. Didolkar,[3] and E.G. Elias[3,4,*]

SUMMARY

Twenty-seven patients with advanced colorectal carcinoma who had earlier failed to respond to 5-fluorouracil (5-FU) were given methyl-CCNU. Of the 21 patients who received methyl-CCNU alone, two (9.5%) responded partially with a mean duration of 28 weeks. Of the six patients who received methyl-CCNU and β-TGdR, three had a partial response. There were no complete responses in either group. Response was not related to performance status, site of metastasis, or previous response to 5-FU treatment. It seems that the addition of β-TGdR to methyl-CCNU might give better results after failure to previous 5-FU treatment. Hematologic toxicity was noted in 33% of the patients and gastrointestinal toxicity in 25%.

[Cancer Treat Rep 60:235-238, 1976]

Of all the chemotherapeutic drugs used in the treatment of advanced colorectal cancer, 5-fluorouracil (5-FU) seems to be the most widely used. The usual objective response rate is approximately 25% (1,2). Since patients with advanced colorectal carcinoma, compared to patients with some other cancers, live relatively longer even after the failure to 5-FU, there is a need for other effective chemotherapeutic agents and other modalities of therapy.

In 1973 Moertel reported effectiveness of methyl-CCNU in advanced colorectal cancer (3). He also suggested that the previous use of cytotoxic agents resulted in an unfavorable responsiveness to the nitrosoureas, although in his study, previous 5-FU treatment had minimal effect on the response to nitrosoureas. This encouraged us to use methyl-CCNU in 27 patients with advanced colorectal carcinoma who had previously failed on 5-FU treatment.

MATERIALS AND METHODS

From October 1973 to May 1975 a total of 27 patients with advanced colorectal cancer were treated with methyl-CCNU after having failed prior 5-FU treatment. Of these, 21 patients received methyl-CCNU alone and six received methyl-CCNU and β-TGdR.[5] In five of the 21 patients treated with methyl-CCNU alone, previous 5-FU treatment had been combined with either β-TGdR or actinomycin D and cyclophosphamide.

The age range was 27-84 years with a median of 58 years. There were 14 men and 13 women. All had histologically proven adenocarcinoma of the colon or rectum and had distant metastasis. Thirteen patients had metastasis to the lung, 16 to the liver, and seven to other sites. In addition, six patients had locally recurrent, unresectable disease. Fourteen patients had measurable disease and 13 had evaluable disease. Of the 27 patients, two (7.4%) had had a partial response to 5-FU before they failed such therapy. Radiation therapy to the perineum had been given in two instances for locally recurrent disease without any response, and in both cases it was stopped at least 6 months prior to the administration of methyl-CCNU. The Eastern Cooperative Oncology Group (ECOG) performance status[6] was 0 in six patients, 1 in 14, 2 in

[1]Received Oct 1, 1975; revised Jan 9, 1976; accepted Jan 19, 1976.

[2]Methyl-CCNU: CAS reg. No. 33073-59-5; urea, 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitroso-.

5-Fluorouracil: CAS reg. No. 51-21-8.

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[5]β-TGdR: NSC-71261; 9*H*-purine-6-thiol, 2-amino-9-(2-deoxy-β-D-erythro-pentofuranosyl)-, monohydrate.

[6]ECOG performance status: 0 = normal; 1 = symptomatic but ambulatory; 2 = in bed <50% of time; 3 = in bed > 50% of time; 4 = in bed 100% of time.

three, and 3 in four. There were no patients with performance status 4.

Methyl-CCNU alone was given orally to 21 patients at a dose of 175-200 mg/m^2. The drug was repeated every 6 weeks. The other six patients received methyl-CCNU orally at a dose of 130 mg/m^2 repeated every 8 weeks and β-TGdR (60 mg/m^2) iv for 5 consecutive days every 4 weeks.

All patients had complete physical examinations, chest roentgenograms, blood cell counts, and 12 profiles of blood chemistries (including blood urea nitrogen, alkaline phosphatase, serum glutamic oxaloacetic transaminase [SGOT], bilirubin, lactic dehydrogenase, total serum protein, and fasting blood sugar) before the treatment was given. In addition, many patients had liver scans and other radiologic examinations pertaining to their metastases.

All patients were evaluated every 4-8 weeks at which time the hematologic and biochemical analyses were repeated. All signs and symptoms of toxicity were recorded.

RESULTS

The response was recorded in the following way: *complete response* = complete disappearance of disease for at least 1 month; *partial response* = decrease by at least 50% in the sum of the product of two perpendicular diameters of the measurable lesions or a decrease by at least 50% of the evaluable disease, lasting for at least 1 month with no new lesions or progression in any other nonmeasurable disease; *static disease* = decrease by <50% in the sum of the products of two perpendicular diameters of the measurable lesions or a decrease by <50% of evaluable disease lasting for at least 1 month with no new lesions or progression of nonmeasurable disease; *progression* = increase ≥ 25% in the sum of the products of two perpendicular diameters of the measurable lesions and/or development of new lesions or progression of evaluable disease.

Hepatomegaly was considered measurable disease when the liver was palpable at least 5 cm below the right costal margin. For a response to be recorded, there had to be a reduction of at least 50% in the sum of the measurements below the xiphoid and the costal margins at the midclavicular lines. In addition, the liver function tests which included SGOT, bilirubin, and alkaline phosphatase must have remained stable or improved.

RESPONSE

Of the 21 patients who received methyl-CCNU alone, two (9.5%) responded partially, six had static disease, and 13 progressed. In the latter group are included two patients who died before they could be evaluated. Since these patients died in other hospitals and no autopsies were performed, the exact cause of death could not be determined.

Both the responders had measurable disease and the duration of response was 28 weeks in both instances. The response was seen after 6 weeks from the first administration of methyl-CCNU in both patients, ie, at the time the patients received the second dose of the drug. In patients categorized under static disease, the condition remained stable from 6 to 30 weeks with a median of 14.5 weeks.

Survival of the two responders was 7 and 13 months following administration of methyl-CCNU. The median survival was 2 months for the patients who progressed and 5 months for those with static disease. Response in relation to performance status is presented in table 1.

None of the methyl-CCNU responders had responded to prior 5-FU treatment (table 2).

TABLE 1.—Response to methyl-CCNU in relation to performance status

Response to methyl-CCNU	No. of patients	No. of patients with performance status—				
		0	1	2	3	4
Complete	0	0	0	0	0	0
Partial	2	0	2	0	0	0
Static disease	6	1	3	2	0	0
Progression	13	3	6	1	3	0
Total	21	4	11	3	3	0

TABLE 2.—Response to methyl-CCNU in relation to previous response to 5-FU

Response to methyl-CCNU	No. of patients	5-FU treatment: No. of patients with—			
		Complete response	Partial response	Static disease	Progression
Complete	0	0	0	0	0
Partial	2	0	0	0	2
Static disease	6	0	0	4	2
Progression	13	0	2	9	2

Of the six patients who received methyl-CCNU and β-TGdR, three responded partially, one had static disease for > 7 weeks, and two progressed. The duration of response was > 6 weeks in two patients and 15 weeks in one patient.

TOXICITY

Of the 21 patients who received methyl-CCNU alone, only 19 were evaluable for toxicity because two patients died before the toxicity could be assessed. It would be difficult to relate these two deaths to drug toxicity, disease progression, or both.

The most common toxicity was hematologic, in the form of leukopenia (white blood cell count [wbc] <4000 cells/mm^3) in six patients and thrombocytopenia (platelet count <100,000 cells/mm^3) in seven patients. Of these patients, four had both leukopenia and thrombocytopenia. The range of wbc count was 400-3700 cells/mm^3 with a median value of 2200, while the range of platelet count was 2500-96,000 cells/mm^3 with a median value of 54,000.

Both responders had leukopenia, and one, in addition, had thrombocytopenia. In most cases hematologic toxicity appeared after the second course of methyl-CCNU.

Gastrointestinal toxicity, in the form of nausea and vomiting appearing from 2 to 24 hours after administration of the drug, was noticed in five patients. No signs of alopecia or renal, hepatic, or neurologic toxicity were recorded in this study.

In the other group of patients who received methyl-CCNU and β-TGdR, one patient died before toxicity could be evaluated. Again, it would be difficult to relate the death to drug toxicity, progression of disease, or both. Of the remaining five evaluable patients, three had hematologic and one had gastrointestinal toxicity.

DISCUSSION

Biologically, colorectal carcinoma is a relatively slow-growing cancer. Patients with advanced colorectal carcinoma have a median survival of 7 months (4). 5-FU has been the most widely used chemotherapeutic agent for the last 15 years in the treatment of this tumor. Regardless of how this drug is administered, the rate of response is low and the duration is usually limited to a few months (1). This situation indicates a need for other chemotherapeutic agents and other modalities of treatment in the management of this disease.

Methyl-CCNU has shown activity against Lewis lung carcinoma in mice (5). This is a slow-growing tumor with growth kinetics which resemble those of colorectal carcinoma. Moertel's studies with nitrosoureas (3) revealed activity of methyl-CCNU against colorectal cancer comparable with 5-FU.

We had a sizable number of patients with advanced colorectal carcinoma who had failed previous 5-FU treatment. These patients were treated with methyl-CCNU alone, and a few received the combination of methyl-CCNU and β-TGdR. The response rate was 9.5% when methyl-CCNU was given as a single agent; there were no complete responders. The response rate is comparable to the overall response rate of 8% reported in the phase II trial of the Southwest Oncology Group (6), and to the 12% rate of Moertel's series of patients who had other chemotherapy prior to nitrosourea administration (3,7).

The response rate has been reported to be 26% when methyl-CCNU was given as a first drug to patients with advanced colorectal carcinoma (3). When it was given after 5-FU or other chemotherapeutic agents, the response rate was 12.5% in Moertel's series (3) and 9.5% in our series. In this study, the response to methyl-CCNU was not related to previous response to 5-FU treatment. On the

contrary, both responders to methyl-CCNU had never responded to prior 5-FU therapy. In the same way, the responders to prior 5-FU treatment in our group progressed on methyl-CCNU. This could indicate that methyl-CCNU might affect clones of cells different from those affected by 5-FU, and a combination of the two drugs might offer an additive effect.

Methyl-CCNU has been tried in combination with cyclophosphamide.[7] There were three responses among 11 patients with colorectal carcinoma (8) and two responses among 25 patients with gastrointestinal adenocarcinoma (9). Horton and his associates have reported 6-thioguanine[8] to be effective in colorectal cancer (10). The β-anomer, β-TGdR, has been shown to be superior to 6-thioguanine in C3H mammary tumors in mice (11). Since the response rate of methyl-CCNU was low in our series, a small number of patients with advanced colorectal cancer, comparable with the group of patients who received methyl-CCNU alone, were treated with a combination of methyl-CCNU and β-TGdR. Although this was not a randomized study between methyl-CCNU alone and methyl-CCNU plus β-TGdR, the response obtained was much higher in the combination chemotherapy group (three of six patients) than in the methyl-CCNU alone group (two of 21 patients). This could indicate that a combination of methyl-CCNU and β-TGdR or other chemotherapeutic agents might give a better response rate.

It should be noted that the response to methyl-CCNU was not related to performance status in our series. However, the patients who had ECOG performance status 3 showed progression of disease during methyl-CCNU treatment.

Duration of response to methyl-CCNU alone was 6.4 months in our group of patients, which was comparable to other series (8).

The hematologic toxicity of methyl-CCNU is well documented (9). The SWOG, in their phase II study (6), reported leukopenia with wbc counts <4000 cells/mm^3 in 66% of their patients and thrombocytopenia with platelet counts <100,000 cells/mm^3 in 67%, while Moertel observed leukopenia and thrombocytopenia in 69% and 47% respectively (3). In our study, we found leukopenia in 37% and thrombocytopenia in 32%. It should be pointed out that Moertel (3) used a higher dosage of methyl-CCNU. Furthermore, the blood cell counts of some of our patients were not available at 3 weeks after receiving the drug. Hematologic parameters usually returned to normal within 2 weeks. Gastrointestinal toxicity appeared in 26% of our patients as compared to 60% and 43% reported by the SWOG and Moertel respectively.

Although hepatic and neurologic toxic effects after methyl-CCNU treatment have been reported (6,9), they were not encountered in this study. The toxicity seen in the patients treated with methyl-CCNU and β-TGdR seemed to be comparable with the toxicity seen with methyl-CCNU alone.

[7]Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate.

[8]6-Thioguanine: NSC-752; CAS reg. No. 1832-72-0; purine-6-thiol, 2-amino-.

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Vincristine (NSC-67574) in the Treatment of Small-Cell Anaplastic Carcinoma of the Lung[1,2,3]

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SUMMARY

In a phase II trial the effect of vincristine as a single agent was evaluated in patients with small-cell anaplastic carcinoma of the lung. The dosage of vincristine was 1.5 mg/m²/week given iv for 4 weeks, followed by 1.5 mg/m² given every other week with dose modifications according to neurologic tolerance. Nineteen of 27 patients included in the study were evaluable. Objective response was observed in eight patients (42%) including three who were not previously treated and five who were previously treated. The median duration of response was 60 days (range, 21-182 days) with the response always occurring within 4 weeks. This study demonstrates that vincristine is an active agent for small-cell anaplastic carcinoma without cross resistance to CCNU, cyclophosphamide, or methotrexate.

[Cancer Treat Rep 60:239-242, 1976]

Small-cell anaplastic carcinoma accounts for about 25% of all cases of primary carcinoma of the lung (1). Until recently the treatment of this tumor type has been surgical resection or irradiation. The results have been poorer than in other histologic subtypes of lung cancer (1), probably because in a high percentage of cases small-cell anaplastic carcinoma has disseminated at the time of diagnosis, evidenced, for example, by examination of the bone marrow (2).

After the introduction of combination chemotherapy against this tumor, objective regression has been seen in 60%-80% of the patients and prolongation of the median survival time has been obtained with combination chemotherapy used either alone or with radiotherapy (3-10).

Vincristine, a vinca alkaloid, has been included in many of the chemotherapy regimens (3-6, 8-10). However, the literature on the antitumor effects of vincristine used as a single agent in small-cell anaplastic carcinoma covers only a few patients (11,12). Therefore, we undertook the present phase II study to determine the effect of vincristine when used as a single agent in the treatment of small-cell anaplastic carcinoma of the lung.

MATERIALS AND METHODS

This study comprised 27 patients with small-cell anaplastic carcinoma of the bronchus treated during the period from October 1973 to February 1975. All had histologically verified small-cell anaplastic carcinoma according to the World Health Organization's classification (13). Eight patients were excluded because they died within 1 week after the start of treatment.

Requirements for entry into the study included white blood cell (wbc) counts > 3000 cells/μl and platelet counts > 100,000 platelets/μl unless low counts were disease-related due to bone marrow infiltration by tumor cells. For this reason, a bone marrow biopsy and aspiration were performed in all patients.

A prerequisite for inclusion in the study was measurable disease, ie, palpable metastases or radiologic chest lesions. Chest roentgenograms and clinical evaluation, including hematologic parameters, were performed at least twice monthly. Regression was defined as at least a 50% reduction in the product of the longest and the widest perpendicular diameters of measurable tumors, without the progression of other tumors or the emergence of new tumors.

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[2]Supported by grant 512-3057 from the Danish Medical Research Council and by the Esper and Olga Boel's Foundation.

[3]Vincristine: CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1).

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TABLE 1.—Results in 19 patients with small-cell anaplastic carcinoma of the lung treated with vincristine alone

Patient No.	Age (sex)	Previous treatment	Duration of vincristine treatment (days)	Partial response	Day of response	Duration of response (days)	Degree of neurotoxicity*
1	55(F)	CTX, CCNU, MTX, and irradiation	224	+	27	182	2
2	71(M)	None	37				2
3	66(M)	Irradiation	32				2
4	71(M)	None	21				2
5	70(M)	CTX, CCNU, MTX, and irradiation	114	+	30	77	2
6	66(M)	CTX, CCNU, MTX, and irradiation	46				2
7	74(M)	None	250				1
8	65(M)	CTX, CCNU, MTX, and irradiation	35				0
9	72(M)	None	80	+	19	60	0
10	47(M)	CTX, CCNU, MTX, and irradiation	35				0
11	61(F)	CTX, CCNU, MTX, and irradiation	41	+	8	60	1
12	71(M)	None	36	+	15	21	2
13	64(M)	Irradiation	49	+	14	29	2
14	58(M)	None	43				2
15	61(F)	CTX, CCNU, MTX, and irradiation	24				1
16	51(M)	Irradiation	67				1
17	71(M)	None	70	+	8	62	2
18	59(M)	CTX, CCNU, MTX, and irradiation	49	+	9	41	2
19	54(M)	CTX, CCNU, MTX, and irradiation	35				0

*For definition of degree of neurotoxicity *see* Materials and Methods section.

The vincristine dose was 1.5 mg/m²/week given iv for 4 weeks followed by 1.5 mg/m² given every other week with dose modification according to neurologic tolerance.

Vincristine neurotoxicity was graded as follows: grade 0, no signs of neurotoxicity; grade 1, paresthesias, numbness, decreased deep tendon reflexes, and alopecia; grade 2, moderately slapping gait or foot drop, wrist drop, severe weakness, and constipation; grade 3, severe atrophy, inability to walk, persistent wrist or foot drop, and ileus requiring tube or surgery; grade 4, death. With grade 0 toxicity no dose reduction took place, with grade 1 toxicity 50% of the scheduled dose was given, and with grade 2 or 3 toxicity the medication was stopped. Treatment with vincristine was otherwise continued until progression of disease occurred.

Of the 19 evaluable patients nine had been previously treated with a three-drug combination (consisting of CCNU, cyclophosphamide, and methotrexate) plus irradiation to the primary tumor and mediastinum (4000 rads). Three patients had received irradiation only and seven patients had not been previously treated.

RESULTS

Age, sex, previous treatment, and results of treatment with vincristine are summarized in table 1.

Eight (42%) of 19 patients obtained partial response, including three previously untreated and five previously treated patients; if the eight nonevaluable patients with early deaths are included the response rate drops to 30%. In one patient the response was observed on chest roentgenograms; in the remaining seven it was seen in palpable metastatic lesions. The median day for start of remission was Day 15, and the median duration of the remission was 60 days (range, 21-182 days). Furthermore, one patient had no tumor progression in 250 days.

Toxic effects included gastrointestinal and neuromuscular complications, as well as alopecia. Ten of 19 patients had gastrointestinal complications ranging from vomiting to ill-defined abdominal discomfort and constipation. Ileus did not occur. Fifty-eight percent (11 of 19) of the patients had neuromuscular complications which included a decrease or loss of deep tendon reflexes that started in the lower limbs. Muscular weakness in the form of drop foot occurred in one patient (No. 1); it regressed slowly and incompletely after the medication was stopped. Hematologic toxic effects, defined as a decrease of > 25% of pretreatment values of wbcs and/or thrombocytes, were not observed in any patients.

The mean vincristine dose in the evaluable patients for the first 4 weeks was 100%, 92%, 88%, and 83% of the values calculated according to the protocol.

DISCUSSION

This study demonstrates that the vinca alkaloid vincristine is a fairly active agent in small-cell anaplastic carcinoma of the lung with an overall response rate of 42%. The responses induced by vincristine occurred rapidly but were usually of short duration.

Direct comparison with other studies using vincristine as a single agent in the treatment of small-cell carcinoma is difficult because subdivision into the different cell types of lung cancer (in particular, anaplastic carcinoma) is rarely attempted. However, it is noteworthy that in 1964 Shaw and Bruner (12) observed objective improvement in two of four patients with small-cell anaplastic carcinoma treated with vincristine while the overall response rate in bronchogenic carcinoma has been < 10% (11,12,14). In addition, the other vinca alkaloid, vinblastine,[5] has also proved active against small-cell anaplastic carcinoma, but again the number of patients treated has been rather small and the overall response rate in bronchogenic carcinoma has been modest (15-23).

In the present study it is of interest that tumor regression occurred in previously untreated as well as previously treated patients. The latter category included four patients who had progressed while receiving radiotherapy plus cyclophosphamide, CCNU, and methotrexate, indicating lack of cross resistance of vincristine to these compounds. This fact, and the lack of bone marrow toxicity, makes vincristine a useful agent in the combination chemotherapy of small cell anaplastic carcinoma.

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BCNU (NSC-409962) and Procarbazine (NSC-77213) Treatment for Malignant Brain Tumors[1,2,3]

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SUMMARY

Sixty-five patients with malignant brain tumors were treated with a combination of BCNU (100 mg/m^2 qd × 1) and procarbazine (100 mg/m^2 qd × 14); the cycle was repeated in 1 month and then on a 6-week schedule with procarbazine being given for 21 days. Forty-five patients had malignant gliomas (glioblastoma multiforme, anaplastic astrocytoma, malignant glioma, or gemistocytic astrocytoma) and were evaluated as a group. All patients had either shown evidence of tumor regrowth after previous surgery and/or radiotherapy, or had deep unbiopsied tumors presumed to be malignant gliomas. Of these 45 patients, 13 of 45 (30%) were judged to be unequivocal responders and an additional eight of 45 (17%) were designated as probable responders. The median duration of clinical response was 34 weeks for responders and 20 weeks for probable responders. The combination of BCNU and procarbazine, therefore, was somewhat inferior to a previous combination of procarbazine, CCNU, and vincristine.

[Cancer Treat Rep 60:243-249, 1976]

In this study BCNU and procarbazine, two of the most effective agents in brain tumor chemotherapy, were used in combination therapy. As single agents these drugs have shown activity against primary brain tumors, particularly malignant gliomas (1-3). BCNU alone achieved a response rate of approximately 50% for a median duration of 9 months in patients with recurrent (ie, previously treated) brain tumors, while a response rate of 50% for a median duration of 6 months was reported for procarbazine (4). In a previous study we gave procarbazine, CCNU, and vincristine (PCV) in combination for recurrent malignant gliomas and achieved a response rate of 60% for a median duration of 9 months (5).

Since we found BCNU to be somewhat more active than CCNU (4), we hoped that a combination of BCNU and procarbazine would be superior to PCV. Vincristine has shown limited activity against malignant gliomas in clinical and laboratory studies (6-8), and we believed that the deletion of vincristine from this study was justifiable on the basis of simplifying the treatment schedule.

The goals of this study were (*a*) to evaluate the effectiveness of BCNU and procarbazine in combination against malignant gliomas; and (*b*) to manipulate the dosage of one of the two agents independently of the other in an attempt to minimize myelotoxicity thereby increasing the length of treatment with effective drug doses.

MATERIALS AND METHODS

Patient Selection

Patients admitted consecutively to the Chemotherapy Service, Department of Neurological Surgery, University of California, San Francisco, who

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[2]Supported by Public Health Service grant CA-13525 from the National Cancer Institute (NCI), National Institutes of Health, Department of Health, Education, and Welfare.

[3]BCNU: CAS reg. No. 154-93-8; urea, 1,3-bis(2-chloroethyl)-1-nitroso-. Supplied by Drug Research and Development Program, Division of Cancer Treatment, NCI.

Procarbazine: CAS reg. No. 491-88-3; *p*-toluamide, *N*-isopropyl-α-(2-methylhydrazino)-, monohydrochloride. Procarbazine capsules (25 mg) were especially prepared and generously supplied by Dr. Edward Miller, Hoffmann-La Roche Inc, Piscataway, NJ (IND No. 10637).

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showed unequivocal tumor progression (ie, increased tumor regrowth after surgery and/or radiotherapy and/or chemotherapy) were eligible for this study. Patients with deep or unresectable tumors were, when appropriate, eligible without prior therapy. The requirements for entry in the study included the following: (*a*) At least 2 months must have elapsed since surgery, at least 3 months since radiotherapy, and a minimum period of 6 weeks since the last dose of any chemotherapeutic agent. (*b*) Clinical neurologic and neuroradiologic evidence of increasing tumor had to be documented. Neuroradiologic evidence was obtained by angiography, isotope brain scintiscan, and computerized axial tomography (CAT) scan. Pneumoencephalography rarely proved to be of more value than these three procedures. (*c*) Immediately prior to entry, hemogram and platelet count, SMA-12, urinalysis, prothrombin time, isotope brain scintiscan, electroencephalogram (EEG), and frequently CAT scan were obtained for each patient. (*d*) Informed consent, as promulgated by the Committee on Human Experimentation of the University of California, was obtained prior to the start of therapy.

Therapy

All patients were initially treated with BCNU (100 mg/m^2 iv on Day 1) and procarbazine (100 mg/m^2 orally on Days 2-15) followed by a 2-week rest period. This schedule was repeated on Day 29 (4 weeks after the first injection of BCNU). Six weeks after the start of the second course, BCNU and procarbazine were again given at the same dosages; however, procarbazine was given for 3 weeks (Days 2-22) followed by a 3-week rest period. This schedule was repeated until the tumor showed progression, either with or without initial regression, and at this point therapy was stopped. Laboratory studies throughout therapy included a complete blood cell and platelet count twice weekly, and isotope brain scintiscan, EEG, CAT scan, clinical neurologic examination, and SMA-12 every 6 weeks.

Dose adjustments were made for myelotoxicity by either (*a*) reducing the doses of both BCNU and procarbazine an equal amount (rather than increasing the interval between courses) (table 1), or (*b*) reducing the dose of either drug. Patients were randomized between these two dose-reduction schedules. In most cases, patients received the first two courses of therapy at the full dose outlined before a reduction was necessary.

Dexamethasone doses were very carefully adjusted as medically indicated to improve the level of neurologic function and to reduce headache and nausea. Aware that the improvement caused by glucocorticoids can mimic the response to cytotoxic drug therapy, we increased dexamethasone doses only when required by marked clinical deterioration. At 4-6-week intervals we attempted to reduce the dexamethasone dose if the patient was improving.

Because of the monoamine oxidase-inhibiting action of procarbazine, diet was restricted to avoid high tyramine-containing foods such as aged cheese, alcohol, and bananas. Anticonvulsants and antiemetics were used as medically indicated.

TABLE 1.—Dosage-modification scale used in the study of BCNU and procarbazine when both drugs were reduced by equal amounts

Nadir counts between courses		Dose of BCNU and procarbazine (mg/m^2)
Wbc (cells/mm^3)	Platelet (cells/mm^3)	
≥ 4000	≥ 100,000	100
3000-4000	75,000-100,000	75
2000-3000	50,000-75,000	50
1500-2000	20,000-50,000	25
< 1500	< 20,000	0

EVALUATION OF THERAPEUTIC RESPONSE

Of the 65 patients entered in this study, ten were excluded from analysis because they either died (eight patients) or were lost to followup (two patients) before receiving a second course of therapy. Five of the eight deaths occurred in patients with malignant gliomas. Those patients who died before receiving a second course of therapy died from far-advanced disease or from unrelated causes (ie, pulmonary emboli). It could be argued that eliminating these patients biased the study in favor of slower growing, less aggressive tumors; however, the main goal of the study was to evaluate BCNU and procarbazine therapy in adequately treated patients, and we believe that eliminating this group from the analysis was justifiable.

Response was defined (as in previous studies) as unequivocal improvement in both clinical neurologic status and isotope brain scintiscan while receiving a stable or decreasing dose of dexamethasone. For example, when a patient required steroids immediately prior to or during a dose of chemotherapy, the response would not be evaluated until

the patient showed improvement at two consecutive visits on the original or lower steroid dose; or unless steroid therapy was stopped after several days and the patient later improved in terms of clinical neurologic status and isotope brain scan.

Patients designated probable responders showed improvement in either the clinical neurologic examination *or* the isotope brain scintiscan without deterioration in the other category while receiving a stable or decreasing dose of dexamethasone. Those patients who showed neither improvement nor deterioration in both parameters for > 3 months were also included in the probable-response group if they were receiving a stable or decreasing dose of dexamethasone. The no-response category included patients who received at least two courses of therapy but showed disease progression in < 3 months. At least two courses of therapy were required to evaluate a patient for response.

Table 2 summarizes the specific tumor diagnoses, responses, and evaluability for all patients in the study. Statistical analysis, however, is based only on the 45 evaluable patients who were entered into the category of malignant glioma which specifically included glioblastoma multiforme, malignant astrocytoma, low-grade astrocytoma (these patients were included because their clinical presentation at the time of therapy was consistent with features of a malignant tumor), mixed glioma, and presumed glioma (unbiopsied).

In the category of malignant glioma there were 13 responders, eight probable responders, and 24 nonresponders. This resulted in a response rate of 30%, or 47% if the response and probable-response patients were combined. Table 3 shows the duration of response and probable response (in wks) for all tumor types. For the malignant gliomas, the median duration was 34 weeks for responders and 20 weeks for probable responders.

Of the patients with malignant glioma, 16 received no previous surgery (except for three who had biopsy only) and no radiotherapy. The results are summarized in table 4. Interestingly, this group did nearly as well as those receiving previous surgery and/or radiotherapy, with a response rate of 31% and a probable response rate of 18%. The median duration of response was 28 weeks, slightly less than the 35-week median duration for the group of patients who had received some form of previous therapy (no statistical difference was found by the Wilcoxon rank sum analysis).

TABLE 2.—Classification of all patients treated with the BCNU and procarbazine combination by tumor type, evaluability, and response

Tumor type	Total No. of patients treated	No. of unevaluable patients	No. of patients with— Response	Probable response	No response
Malignant glioma					
Glioblastoma multiforme	18	5	3	3	7
Malignant astrocytoma	17	2	5	2	8
Low-grade astrocytoma*	6	0	1	1	4
Mixed glioma	2	0	0	1	1
Presumed glioma (unbiopsied)†	9	0	4	1	4
Subtotal	52	7	13	8	24
Others					
Ependymoma	3	0	1	1	1
Oligodendroglioma	2	0	1	0	1
Brain stem glioma	5	1	0	0	4
Metastatic	3	2	0	0	1
Subtotal	13	3	2	1	7
Total	65	10	15	9	31

*Originally diagnosed as low-grade glioma; however, the course of disease at the time of therapy was that of malignant glioma.

†Of the original 15 unbiopsied patients, 6 have been autopsied (to date) and were found to have malignant gliomas.

TABLE 3.—BCNU and procarbazine duration of response and probable response by tumor type for individual patients

Tumor type	Duration (wks) of—	
	Responses	Probable responses
Glioblastoma multiforme	17, 28, 30	14, 16, 23
Malignant astrocytoma	34, 41, 43, 63, 73†	18, 22
Low-grade astrocytoma*	23	17
Mixed glioma	—	23
Presumed glioma (unbiopsied)	17, 22, 36, 69	23
Ependymoma	22	11
Oligodendroglioma	30	—

*Originally diagnosed as low-grade glioma; however, the course of disease at the time of therapy was that of malignant glioma.
†This patient continues to receive this therapy.

Myelotoxicity was the major side effect of this therapy. Twenty-three malignant-glioma patients had dose reductions (table 5) due to depressed white blood cell (wbc) and/or platelet counts. In 15 of these patients, the severity of the toxic effects warranted discontinuation of therapy or a reduction to 25% of the original dose. Since myelotoxicity from BCNU commonly occurs from 3 to 6 weeks after administration and myelotoxicity from procarbazine occurs at variable and unpredictable times, overlapping toxicity between courses was common. Because of this, the time to bone-marrow recovery was difficult to predict and to analyze. Dose adjustments were based on the nadir wbc and/or platelet counts in the interval between courses. There was one death in which myelotoxicity was almost certainly a contributory factor. This patient had a wbc count of 300 cells/mm^3 and a platelet count of 16,000 cells/mm^3 after the second course of therapy and died during the 7th week of the study.

TABLE 4.—Patients treated with BCNU and procarbazine who did not receive any previous therapy

Condition	Total No. of patients	No. of patients with—	
		Response	Probable response
No surgery or radiotherapy	13	3 (17,22,69 wks)*	2 (16,23 wks)*
Biopsy but no radiotherapy	3	2 (28,43 wks)*	0
Total	16	5(31%)	2(18%)
Median duration of response		28 wks	19.5 wks

*Response durations of individual patients.

TABLE 5.—Time of actual dose adjustments for malignant gliomas

Time of first dose reduction (course)	No. of patients with dose reductions of—			
	BCNU and procarbazine	BCNU only	Procarbazine only	Total
2nd	2	2	4	8
3rd	7	1	3	11
4th	2	0	0	2
5th	1	0	1	2

In this group of 45 patients there were 26 men and 19 women and the age range was from 24 to 71 years. Sixteen patients (36%) had not received previous therapy for their disease, four (9%) had had surgical resection, five (11%) had received radiotherapy, and 20 (44%) had undergone both surgical resection and radiotherapy before entry into this study. No statistical correlation was apparent in comparing frequency of response to sex, age, or prior therapy.

The time from first symptoms of brain tumor (eg, original tumor symptoms) until treatment on this protocol ranged from 5 to 367 weeks. The median was 47 weeks for those patients who responded and 38 weeks for the nonresponders. By the Wilcoxon rank sum analysis (9) and the likelihood function of Breslow (10), no relationship was found between the duration of symptoms prior to chemotherapy and the response to BCNU and procarbazine.

Figure 1 is an exponential fit of all malignant-glioma patients who were still in the study 5 weeks or more after therapy was started. It shows the time to cessation of therapy because of tumor progression. The criteria for stopping therapy included unequivocal deterioration in both clinical neurologic status and isotope brain scan with or without a concomitant increase in dexamethasone dosage. The median length of time to discontinuation of therapy was 16 weeks for all patients.

In figure 2, patients are divided into the categories of response, probable response, and no response, and the duration of therapy is shown for each group. By the Kaplan-Meier technique for nonparametric estimation from incomplete observations and covariance analysis of censored survival data (11), these groups were found to differ statistically ($P < 0.001$).

Figure 3 summarizes the relationship of the mean total dose per week per course of therapy to the length of response. Included on the graph are

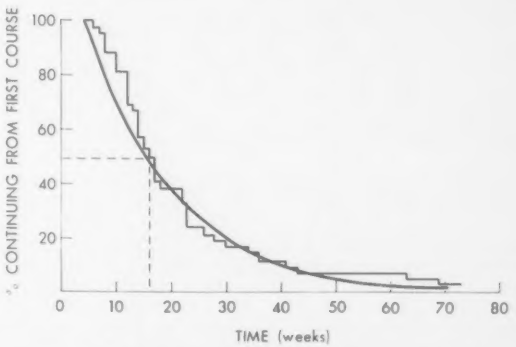

FIGURE 1.—Patients with malignant gliomas who were still receiving BCNU and procarbazine > 5 wks after therapy was started. Sixteen wks was the median period for patients continuing on therapy.

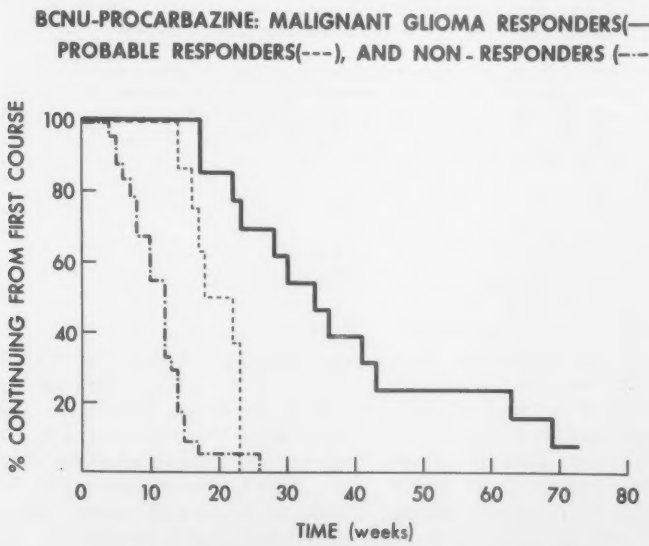

FIGURE 2.—Kaplan-Meier representation of all patients with malignant gliomas treated with BCNU and procarbazine.

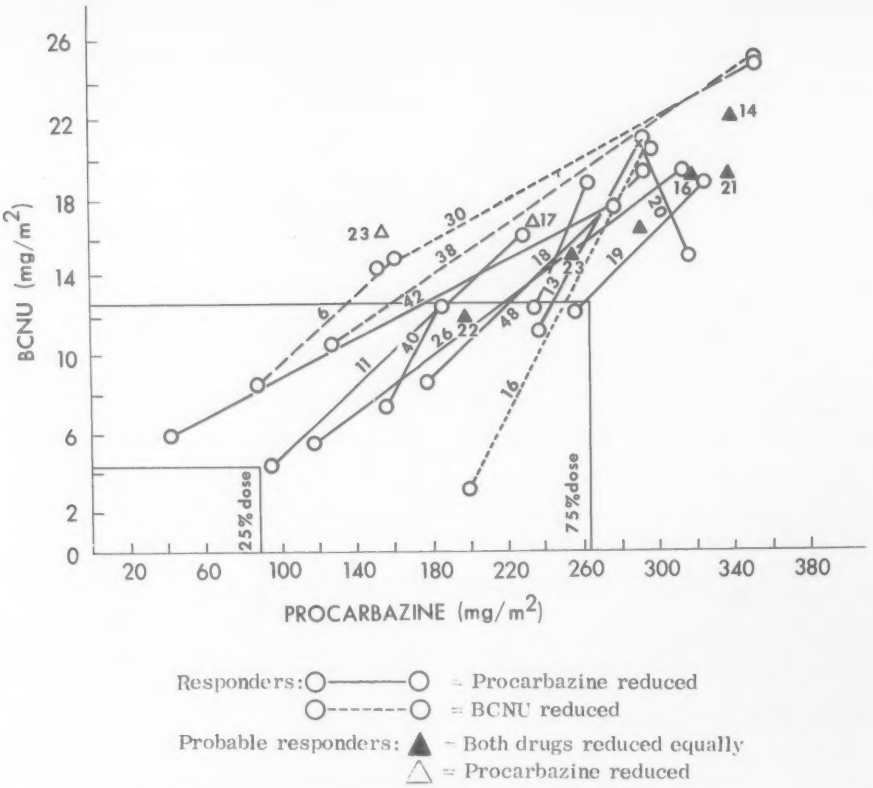

FIGURE 3.—Relationship of BCNU to procarbazine dose in those patients who responded and those who were probable responders. The Nos. associated with the lines and symbols represent the No. of wks of response from the time when the determination of response or probable response was made. The dose was calculated by taking the average of the dose/wk/course of therapy. Those lines designating 25% and 75% dose were calculated on the basis of therapy given every 6 wks.

the calculated doses for courses of therapy repeated every 6 weeks at 75% and at 25% of the original dose (assuming equal reduction in both drugs). Invariably, tumor regrowth was associated with dose reduction. Study results suggest that those patients who had an independent reduction in procarbazine dose showed longer responses than those who had a reduction in BCNU dose. This correlates well with our studies of these two drugs as single agents in the chemotherapy of recurrent malignant gliomas where BCNU was found to be more effective than procarbazine in prolonging remission (4).

DISCUSSION

The combination of BCNU and procarbazine for chemotherapy of recurrent malignant gliomas has shown no advantage over each agent used alone and is somewhat inferior to the combination of PCV. Both the frequency and duration of responses are less than those of the three previous therapies (BCNU, procarbazine, and PCV). We found the response rates and median durations of response to be 51% and 9 months for BCNU, 50% and 6 months for procarbazine, and 60% and 9 months for PCV.

From our experience with this therapy and with the PCV combination it is also clear that the length of remission can be increased only if myelotoxicity is minimized. In both studies, in spite of myelosuppression we reduced the drug dose instead of increasing the interval between courses. Further evaluation of this aspect of therapy is desirable in terms of future combinations of the nitrosoureas with procarbazine.

Since this study of BCNU and procarbazine has again suggested that BCNU is the more effective agent, in future studies the dose of BCNU might be increased and the dose of procarbazine reduced, with the hope of minimizing myelosuppression. Current studies in our Experimental Chemotherapy Laboratory reinforce this approach. After a single administration of BCNU at 30 mg/kg, procarbazine at 10 mg/kg qd × 5 was as effective as 20 mg/kg qd × 5 in C57BL/6 mice with ic Glioma 26; in the same experiment BCNU at 20 mg/kg was not as effective. The LD10 (qd × 5) for procarbazine is $\simeq$200 mg/kg.

In spite of the disappointing results of this current study, we believe it is premature to discard the combination of a nitrosourea and procarbazine. We hope other investigators will pursue different schedules of these two agents in the treatment of malignant gliomas.

[6]Levin VA. Unpublished observations, 1975.

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Response to Medroxyprogesterone Acetate (NSC-26386) as Secondary Hormone Therapy for Metastatic Breast Cancer in Postmenopausal Women[1,2]

David J. Klaassen,* Edna F. Rapp, and Wolfgang E. Hirte[3]

SUMMARY

We have treated 40 postmenopausal women with documented metastatic breast cancer with medroxyprogesterone acetate. The average age was 63 years and the patients were, on the average, 14 years' postmenopausal. Only two patients had received no prior additive hormone therapy. The remainder had previously received estrogen, androgens, or both. Only two patients had objective evidence of tumor regression. In one patient a metastatic node disappeared for 7+ months, and the other patient had well-documented clinical improvement and control of brain metastases for 22 months. Two other patients had mixed responses of chest wall metastases (regression of some but not all lesions), lasting 3 and 4 months respectively. Five other patients had obvious subjective benefit. There were four objective responses (10%) and five subjective responses (12%). There was no correlation between route of administration and response. All patients receiving benefit had previously responded to other hormones. Side effects were usually absent or consisted of mild fluid retention; however, four patients had disease stimulation from therapy.

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Additive hormone therapy has traditionally been the initial and most important treatment for metastatic breast cancer in postmenopausal women. Estrogens have usually been the treatment of choice, with objective response rates in the range of 30%-37% being reported (1,2). Androgens are also effective but have a lower level of activity with response rates of 20% (1,2). Corticosteroids are useful as well (1,2). However, all of these therapies have some side effects and thus a certain degree of morbidity associated with them.

The search continues for other hormonal agents which will be as effective as the above but less toxic. In the early 1950s, Taylor et al (3), Escher (4), and Gordon et al (5) reported that progesterone was active in the treatment of metastatic breast cancer. Many synthetic derivatives have undergone clinical trial, and the results have been summarized by Stoll (1) and Muggia et al (6). The three most active derivatives appear to be megesterol, norethisterone acetate, and medroxyprogesterone acetate (MPA). Norethisterone acetate has been reported by Edelstyn (7) and others (8-11) to have an objective response rate of 41%. Ansfield et al (12) have reported that megesterol has an objective response rate of 23.3%; MPA has been reported to have response rates varying from 9.3% (13) to 22.0% (6).

MPA is a strong progestational agent. Animal studies have shown it to have 80 times the activity of progesterone (14). It is one of the most active compounds in the suppression of ovulation and pituitary gonadotrophin production in animals (14). Large doses of MPA can lower growth hormone, testosterone, luteotrophic hormone, and serum cortisol levels in man (15). The effect on estrogen levels was variable.

We have used MPA to treat 40 postmenopausal women with metastatic breast cancer who have either not responded to or have relapsed after therapy with estrogens and androgens.

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[2]Medroxyprogesterone acetate: CAS reg. No. 71-58-9: pregn-4-ene-3,20-dione, 17-hydroxy-6α-methyl-, acetate.

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TABLE 1.—Distribution of patients by prior therapy

Therapy	No. of patients
Estrogens	20
Androgens	32
Prednisone	21
Endocrine ablative surgery	10
Chemotherapy	21

TABLE 2.—Distribution of patients by dominant lesion

Site	No. of patients
Visceral	16
Bone	17
Soft tissue	7

MATERIALS AND METHODS

All patients were at least 4 years' and an average of 14 years' postmenopausal. The average age was 63 years. All patients had documented metastatic breast cancer and measurable lesions, determined by either physical or radiologic examination.

Table 1 shows the previous therapy of the 40 patients studied. All but two had received prior therapy.

Table 2 categorizes the patients according to the dominant site of the cancer. Patients previously receiving hormonal therapy had this therapy stopped and were left untreated for at least 4-6 weeks before starting MPA to see if rebound regression would occur.

TREATMENT SCHEDULE

Eighteen patients received a depot preparation of MPA im, in a loading dose of 3.2 g over a 2-week period and then 400 mg im at 2-4-week intervals. Twenty-two patients received the drug orally in a dose of 200-300 mg daily. Patients were considered evaluable only if they had received 6 weeks of therapy. Four patients received < 6 weeks of therapy but were included in the study because it was felt that the drug had caused tumor stimulation and clinical deterioration after it had been started.

RESPONSE CRITERIA

The criteria for evaluating response were those used by the Eastern Cooperative Oncology Group and are as follows:

Complete response.—Complete disappearance of all measurable lesions with no new bone lesions and recalcification of osteolytic lesions.

Partial response.—A decrease of 50% or greater in the sum of the products of the two largest perpendicular diameters of all measurable lesions and/or partial recalcification of osteolytic lesions (regression or no change in osteoblastic lesions).

No change.—A decrease of < 50% or an increase of < 25% of the original measurements of the sum of the products of the two largest diameters of all measurable lesions with no change in the osseous lesions.

Progression.—An increase of 25% or greater of the original measurements of the sum of the products of the two largest perpendicular diameters of all measurable lesions and/or the occurrence of new lesions and/or progression of osteolytic lesions.

Relapse.—The appearance of any new lesions and/or an increase of 50% in the sum of the products of the two largest perpendicular diameters of all measurable lesions compared to the size recorded at the start of therapy following a period of initial no change; an increase of 50% of the sum of the products of the two largest perpendicular diameters of all measurable lesions compared to the minimal size recorded at two measurement periods separated by 1 month following a period of response.

RESULTS

The results were disappointing. Only two of the 40 patients had an objective response using the above criteria. In one patient a metastatic lymph node disappeared for 9 months; the other patient had well-documented clinical improvement and control of brain metastases for 22 months. Two other patients had mixed responses of chest wall metastases (regression of some but not all lesions), lasting 3 and 4 months respectively. Five other patients had obvious subjective response (pain relief) but no objective response. The overall response rate was 22%: four objective responses (10%) and five subjective responses (12%).

SIDE EFFECTS

The drug was remarkably well tolerated with mild fluid retention being reported in five patients. Three other patients with pulmonary metastases had documented deterioration in their respiratory symptoms with an increase in dyspnea not attributable to fluid retention. In one case, the dyspnea improved when the drug was stopped. Another patient died from pulmonary metastases within 2 weeks of receiving two doses of im MPA. The drug had been stopped after two doses because her pulmonary symptoms had become worse. One patient

had a marked increase in bone pain following her 3-g loading dose of MPA. The pain gradually subsided when no further drug was given. There was radiologic evidence of tumor progression concurrent with symptomatic deterioration.

DISCUSSION

Stoll (1) has postulated four possible modes of action of progesterones in controlling breast cancer: (*a*) direct inhibition of tumor growth; (*b*) suppression of pituitary hormone secretion; (*c*) sensitization of the tumor to the effect of estrogens; and (*d*) conversion of estrogens to another steroid. Sadoff et al (15) have shown a definite effect on the pituitary adrenal gonadal axis. Stoll (1) has noted that patients who have evidence of some estrogen effect on vaginal smear are the most likely to respond to treatment with progestational agents and that postmenopausal women have a better chance of responding than do premenopausal women. They have also noted that patients with predominantly local disease respond better than patients with visceral or bone disease (1,6,7,12).

The response rate of 10% in this series corresponds to the objective remission rate of 9.3% reported by Goldenberg (13) for the Cooperative Breast Cancer Group and is less than the response rate reported by other authors (6). Although well tolerated by most patients, we feel some patients, particularly those with extensive pulmonary disease, may have tumor exacerbation with this drug. It is costly when compared to other hormones: 1 month's supply of MPA (300 mg daily) costs the patient $35.00 in Ottawa. Since the response rate with this agent is lower than that achieved with estrogens, MPA is not the drug of choice as initial therapy in postmenopausal women.

Also, in view of the response rate and cost of the drug, we feel MPA is of minimal usefulness as secondary therapy in postmenopausal women with metastatic breast cancer. Probably it should be used only for those patients unable to tolerate estrogens or androgens. As noted previously, megesterol and norethisterone acetate are reported to have better response rates, suggesting that further investigations and randomized studies with these two progestational agents should be undertaken, possibly in comparison with other hormonal agents.

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Comparative Experimental Evaluation of Immunodepressive and Toxic Effects of L-Asparaginase (NSC-109229) From *Escherichia coli* and From *Erwinia carotovora*[1,2]

Brief Reports and Preliminary Communications

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L-Asparaginase from *Erwinia carotovora* (*Er. carotovora*) differs from *Escherichia coli* (*E. coli*) L-asparaginase in that it has a shorter plasma half-life, a fivefold greater L-glutaminase activity, a tenfold greater antiblastogenic effect, and a different immunologic specificity (1-6). This report presents a comparative evaluation of (*a*) the immunodepressive activity on the primary immune response to sheep red blood cells (SRBC), (*b*) the appearance of serum anti-L-asparaginase antibody, and (*c*) the morphologic and functional alterations of liver, pancreas, and small intestine in rats treated with multiple doses of both the enzymes.

MATERIALS AND METHODS

Four groups of 12 male and 12 female Wistar rats (120 g) were treated daily ip (5 times a week) for 3 months with 800 and 3200 international units (IU)/kg of *Er. carotovora* and *E. coli* L-asparaginase. Every 15th day four rats (two males and two females) of each treated group and of the control group were killed. Two of them had been injected with SRBC 5 days before to evaluate the number of gamma M immunoglobulin (IgM)-hemolysin-producing spleen cells (HPFC) (7). In all four animals alkaline phosphatase, amylase, serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase, and bilirubin were determined by routine methods. Anti-L-asparaginase antibody was evaluated in undiluted serum by testing against the solution of 128 IU/ml of each enzyme according to Ouchterlony's slide method, and the liver, pancreas, and small intestine were prepared for microscopic and electron-microscopic examination.

RESULTS

During the treatment six of 24 rats that received 3200 IU/kg/day of *E. coli* L-asparaginase died as did two rats from each of the other treated groups. No sex difference was observed. A significant reduction in body-weight increase was recorded only for the higher dose of the *E. coli* enzyme. Biochemical findings were negative throughout treatment with only SGOT transiently reaching the upper limits of normal range on the 15th day. With both doses of the two enzymes, periportal fatty infiltration and fibroblastic proliferation were observed in the liver of about one quarter of the rats and more frequently in those killed on the 15th or 30th day. Enteric damage occurred in four of the rats treated with the *E. coli* enzyme and pancreas damage occurred in one rat receiving 3200 IU/kg of *Er. carotovora* L-asparaginase. Electronmicroscopy did not add anything to the above-described trend of damage.

Both types of L-asparaginase (table 1) given at the dose of 3200 IU/kg/day for 7 days produced a considerable decrease (about 90%) of antibody-producing cells. Variations of immune response to SRBC in rats treated for 90 days (table 2) indicate that both enzymes severely decreased the number of HPFC. No differences between sexes were observed. The immunodepressive effect was roughly dose dependent and apparently more marked after the *E. coli* L-asparaginase. The immunodepressive effect appeared to diminish on progressing treatment, especially in rats treated with the *Er. carotovora* enzyme. Immunodiffusion studies (table 3) show that (*a*) anti-*Er. carotovora* L-asparaginase antibody appeared somewhat earlier than that elicited by the *E. coli* enzyme and was present in a greater number of animals, (*b*) the lower dose was more immunogenic than the higher one for both

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TABLE 1.—HPFC at Day +5 in rats treated ip with 3200 IU/kg/day of *E. coli* or *Er. carotovora* L-asparaginase from Days −2 to +4 with regard to the time of immunization with SRBC (Day 0)

Group*	Body weight change† (g) (mean ± SD)	Spleen weight (mg)/body weight (g) (mean ± SD)	HPFC/spleen	
			Mean (range, ± 1 SD)	Inhibition (%)
E. coli	+22.9 ± 9.4‡	4.21 ± 1.17‡	7977 (3011-21,133)‡	93.8
Er. carotovora	+19.2 ± 7.9‡	5.23 ± 1.39§	11,939 (4073-34,996)§	90.7
Saline	+40.6 ± 7.4	7.91 ± 1.94	128,926 (21,077-788,606)	—

*Nine female rats were used for each group.
†Between Days −2 and +5.
‡Statistically different from controls at the 0.1% level.
§Statistically different from controls at the 1% level.

TABLE 2.—HPFC in rats given multiple doses of *E. coli* or *Er. carotovora* L-asparaginase, at various times after beginning of treatment*

Days after beginning of treatment	L-Asparaginase inhibition (%) of HPFC compared to controls							
	800 IU/kg/day				3200 IU/kg/day			
	E. coli		*Er. carotovora*		*E. coli*		*Er. carotovora*	
	Male	Female	Male	Female	Male	Female	Male	Female
15	79	71	<10	68	84	81	90	73
30	68	98	94	56	94	98	94	<10
45	96	94	63	48	99	98	93	90
60	<10	71	<10	76	70	<10	64	20
75	<10	86	<10	<10	99	99	<10	<10

*HPFC were assayed 5 days after immunization with SRBC. Each value is from a single rat.

TABLE 3.—Anti-L-asparaginase antibody production in rats treated with multiple doses of *E. coli* or *Er. carotovora* enzyme, at various times after beginning of treatment

Days after beginning of treatment	Antigen	Sera of rats treated with L-asparaginase*							
		800 IU/kg/day				3200 IU/kg/day			
		E. coli		*Er. carotovora*		*E. coli*		*Er. carotovora*	
		Male	Female	Male	Female	Male	Female	Male	Female
15	*E. coli*	−,−	−,−	−	−,−	−,−	−,−	−,−	−,−
	Er. carotovora	−,−	−,−	+	+,+	−,−	−,−	−,+	+,+
30	*E. coli*	+,+	−,+	−,−	−,−	−,−	−,−	−,−	−
	Er. carotovora	−,−	−,−	+,+	+,+	−,−	−,−	+,+	−
45	*E. coli*	−,−	+,−	−,−	−,−	−,−	−,−	−,−	−,−
	Er. carotovora	−,−	+,−	−,+	−,+	−,−	−,−	+,+	−,−
60	*E. coli*	−,−	−,+	−,−	−,−	+,−	−,−	−,−	−,−
	Er. carotovora	−,−	−,+	+,+	+,−	+,−	−,−	+,−	+,−
75	*E. coli*	+,−	−,+	−,−	−,+	+	−	−,−	−,−
	Er. carotovora	+,−	−,−	+,+	+,+	−	−	+,+	+
90	*E. coli*	−,−	0	0	−,−	0	0	−,−	−
	Er. carotovora	−,−	0	0	+,+	0	0	+,+	−

* + = A line of precipitation after immunodiffusion in agarose gel for a single rat; − = absence of precipitation for a single animal; 0 = not done; no precipitation was observed with normal control rat serum.

types of enzymes, and (*c*) the anti-*E. coli* L-asparaginase antibody cross reacted with the *Er. carotovora* enzyme in four of 40 samples and the reverse occurred in only one of 43 cases.

DISCUSSION

Both types of L-asparaginase produced a significant number of deaths (greater with the *E. coli* enzyme) with microscopic evidence of liver and enteric mucosal damage in some of the surviving rats. No deaths or histologic lesions were noted in the controls. Toxic effects appeared rather early in the course of treatment and later disappeared. These results are in general agreement with those of previous research (8-11). Probably because of its longer half-life (in part), the *E. coli* L-asparaginase seems to be slightly more toxic than that from the *Er. carotovora*.

In both the short-term and the multiple-dose experiments a similar immunodepressive activity of both types of L-asparaginase was apparent by the splenic atrophy and reduction of HPFC, indicating an inhibition of humoral antibody production. With progressing treatment a reduction of the immunodepressive activity could be observed. This reduction was slightly more marked with the *Er. carotovora* enzyme. These results confirm those of Ashworth and MacLennan (4) in mice but do not seem to support those of Han and Ohnuma (3) who have stated that based on the inhibition of blastogenesis *Er. carotovora* L-asparaginase should be a superior immunodepressant. The behavior of the immunodepressive activity as well as the general toxicity (in part) of both types of L-asparaginase can be explained by the appearance of a precipitating antibody against the enzyme during its administration. This appearance is more evident with *Er. carotovora* L-asparaginase (32 of 42 rats). In addition, low doses seem more active in this respect than the higher ones with both types of enzymes, probably because of the lower immunodepressive activity.

The similarity of the two L-asparaginase enzymes, with respect to both immunodepression and toxicity, suggests that they can be substituted for each other if an immunologic response arises during clinical usage. The use of *E. coli* L-asparaginase might be more advantageous because it appears less immunogenic and slightly more immunodepressive.

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Dose Fractionation Does Not Prevent Repair of Potentially Lethal Damage Induced by Bleomycin (NSC-125066) In Vivo[1,2]

Brief Reports and Preliminary Communications

Peter R. Twentyman[3,*]

When assaying the surviving fraction of cells after treatment with bleomycin, the measured survival is lower if the assay is carried out soon after the end of drug exposure than if the assay is delayed by several hours. This fact has been observed both for cells treated in vitro (1-3) and for cells treated in vivo (3-6). The phenomenon has been interpreted as indicating the existence of repair of potentially lethal damage (PLD) when subculture is delayed. In a recent study of fractionated treatment with bleomycin of cells in vitro, Barranco[4] has found that increased survival of cells with delayed subculture may be reduced if the exposure to bleomycin is given in two fractions separated by 1-4 hours. Furthermore, a recent clinical report (7) has suggested that continuous infusion of bleomycin may be superior to treatment with a single daily dose. In this report, experiments are described which examine the effect of bleomycin given in various fractionation regimens on the survival of cells in the EMT6 mouse tumor.

METHODS AND MATERIALS

The EMT6 mouse tumor system used in this laboratory has been described previously (3,5). In earlier reports we have described the dose-response curve for tumors of various sizes when assay for surviving fraction was carried out either 2 or 24 hours after bleomycin administration (5). The 2-hour curve for all tumor sizes had reached a plateau at a surviving fraction of about 10% for a dose of 1 mg/kg, and there was relatively little further fall between 1 and 10 mg/kg. For a dose of 4 mg/kg, the mean surviving fractions at various times of assay after drug administration were as follows (3): 30 minutes = 2.10^{-3}; 1 hour = 10^{-2}; 2 hours = 10^{-1}; 6 hours = 1.0; 24 hours = 1.0.

In the present experiments, a total dose of either 4 or 8 mg/kg was given during a single day to groups of three mice. The total dose was given in one to seven equal parts at the time intervals shown in table 1. Assay for surviving fraction of tumor cells was carried out on the next day. Each dose of the drug was given ip in a volume of 0.5 ml of Hank's solution. By using these dose schedules, we were sure that every individual dose increment (except 4 mg/kg in seven fractions) was sufficiently large to reduce the 2-hour survival to the plateau of the dose-response curve, and that the largest single dose (8 mg/kg) was still on the plateau.

RESULTS AND DISCUSSION

The surviving fractions of cells obtained from tumors subjected to the various patterns of drug administration are shown in table 2. It is apparent from these results that, in our system, there is no advantage to be gained in terms of tumor cell survival in giving bleomycin in several fractions during the day. We have recently raised doubts regarding the nature of the effect which has become known as "repair of potentially lethal damage" (3) and these doubts remain unresolved. The available data regarding repair of PLD following bleomycin treatment of cells in vitro are rather confusing. Ray et al (1), using a line of Chinese hamster cells, found that only plateau phase cells could recover, whereas exponential phase cells could not. On the other hand two other recent studies (2,3) have shown repair of PLD in both exponential and plateau phase cells. It would not therefore appear that the effect may be attributed to some process occurring only in the absence of cell cycle progression. It is possible that the effect is an artefact caused in the process of making single-cell suspensions prior to assay for survival. It has, however, been demonstrated to occur in an ascites tumor system (6) where the handling procedures consisted solely of drawing cells from the peritoneal cavity of one mouse and injecting them into another mouse. No enzymes or mechanical disaggregation

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[2]Bleomycin: [2,4'-bithiazole]-4-carboxylic acid, 2'-(2-aminoethyl)-, monohydrate.

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TABLE 1.—Bleomycin administration schedule*

No. of fractions	Time of administration						
	10 AM	11 AM	12 noon	1 PM	2 PM	3 PM	4 PM
1				×			
2	×						×
3	×			×			×
4	×		×		×		×
7	×	×	×	×	×	×	×

*Assay for surviving fraction of tumor cells carried out between 10 AM and noon on the next day.

TABLE 2.—Surviving fractions of cells from tumors subjected to various regimens of bleomycin administration

Experiment	Total dose of bleomycin (mg/kg)	Cell survival for total dose in *n* fractions: *n* =				
		1	2	3	4	7
A	4	1.04(0.12)*	0.89(0.10)	1.03(0.12)	0.92(0.11)	1.13(0.13)
B	8	0.81(0.11)	0.62(0.9)	0.77(0.11)	0.86(0.12)	0.78(0.11)

*Nos. in parentheses = 2 standard errors based on the total colony count for groups of 4 replicate dishes.

procedures were used, thereby reducing the trauma to a minimum. Very recently it has been shown that heating the EMT6 solid tumor after administration of bleomycin can apparently prevent the repair of PLD.[5] This would again suggest that the observed effect is a real phenomenon occurring in the tumor rather than an artefact of the experimental procedures.

It must always be remembered, of course, that although delayed subculture may lead to increased measured cell survival for a number of different drugs (4), this is not necessarily true for all drugs. In vivo at least one may expect that circulatory drug breakdown products could influence the operation of repair processes. Observations regarding modification of PLD repair following bleomycin treatment must not therefore be assumed to apply also to other agents. Furthermore, different processes may occur at different drug dosage levels, and the levels which cells encounter during experimental studies are generally well above those likely to be found in the clinical situation.

From the fractionation studies presented here, however, it would appear that whatever process is occurring during a period of delayed subculture following exposure of cells to bleomycin, additional exposure to the drug during such a period is not able to cause any permanent suppression of the process.

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In Vivo Effects of Phenylalanine Ammonia-Lyase[1,2,3]

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Brief Reports and Preliminary Communications

Phenylalanine ammonia-lyase (EC 4.3.1.a) (PAL) catalyzes the deamination of phenylalanine to cinnamic acid and tyrosine to coumaric acid. This enzyme is known to occur only in plants and fungi. It has been reported that *Rhodotorula glutinis* PAL markedly inhibited growth of murine L5178Y leukemia in vivo (1), producing cures in up to 40% of treated tumor-bearing mice.

This report presents the results of our investigation of the antitumor effectiveness of PAL against the murine leukemias EARAD-1 and L5178Y, and B16 melanoma. We have also determined the effect of PAL treatment on free phenylalanine and tyrosine concentrations in tumor cells, plasma, liver, kidney, and muscle.

MATERIALS AND METHODS

Purified PAL derived from *Rhodotorula glutinis* was purchased from P-L Biochemicals, Inc, Milwaukee, Wis. The specific activity of the enzyme was 0.94 unit/mg of protein. The enzyme was stored at −70°C as a suspension in 60% saturated ammonium sulfate. Before injection, the enzyme was dialyzed against 200 volumes of 0.02 M sodium phosphate (pH 7.4)-0.15 M NaCl buffer for 24 hours with one change. The enzyme was assayed by measuring the rate of formation of transcinnamic acid (2). A unit (IU) of PAL is defined as the amount of enzyme that produces 1 μmol of cinnamic acid per minute at 30°C.

Blood for determination of plasma enzyme and amino acid levels was obtained from mice by the orbital bleeding technique (3). Special care was taken to minimize alterations during the processing of blood and tissue samples (3). Tumor, liver, kidney, and femoral muscle were removed from the animals and immediately frozen at −70°C. Samples were prepared for amino acid analysis by homogenizing in picric acid, and excess picric acid was removed with ethyl acetate and ether (4). Amino acid analyses were performed on a Beckman model 119 amino acid analyzer.

BDF_1 (C57BL/6 × DBA/2) mice (obtained from A. R. Schmidt Co, Madison, Wis) were the hosts for L5178Y leukemia and B16 melanoma. The EARAD-1 leukemia was grown in $B6AF_1$/J (C57BL/6J × A/J) mice. Groups of five or ten female mice weighing 19-22 g were used.

Mice were given either Purina laboratory chow, a complete synthetic diet (obtained from General Biochemicals, catalog No. 170010), or a synthetic diet totally deficient in phenylalanine and tyrosine. The absence of phenylalanine and tyrosine in the deficient diet was confirmed by amino acid analysis.

For the growth of ascites tumors, 4×10^5 EARAD-1 cells or 1×10^5 L5178Y cells were injected ip. For the development of solid tumors EARAD-1 cells were injected sc into the right axillary region. B16 melanoma was developed by transplanting small fragments into the right axillary region. Most transplantable mouse tumors were shown to be infected with the lactate dehydrogenase-elevating virus (LDH virus) (5), which is known to decrease the plasma clearance rate of certain enzymes, eg, asparaginase and glutaminase (3,6). Therefore, to ensure uniform experimental conditions, all mice were infected with the LDH virus (5), usually 3 days before the start of an experiment.

RESULTS

The plasma half-life of PAL in mice bearing the L5178Y tumor was 30 hours. Single injections of 300 IU PAL/kg lowered plasma phenylalanine and tyrosine from pretreatment levels of 60 and 49 nmols/ml respectively to unmeasurably low levels (< 2 nmols/ml) within 4 hours and maintained these low levels for 24 hours. After 48 hours plasma phenylalanine and tyrosine concentrations

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were 3 and 5 nmols/ml respectively. A dose of 100 IU PAL/kg lowered plasma phenylalanine to 6, 3, and 18 and tyrosine to 6, 5, and 25 nmols/ml after 4, 24, and 48 hours respectively. Table 1 describes results of a study designed to test the antitumor effectiveness of PAL on mice bearing the ascites forms of L5178Y or EARAD-1 leukemias. The conditions employed in this study were essentially the same as those described by Abell et al (1), who reported a 43% cure rate with PAL in mice bearing L5178Y leukemia. In the present study, PAL treatment did not significantly increase the median survival time or long-term survival in mice bearing the ascites forms of L5178Y or EARAD-1 leukemias (table 1). Experiments also showed that treatment with PAL for 7 days at a dose of 300 or 600 IU/kg/day did not cause significant increases in median survival time in mice bearing B16 melanoma whether treatment was started on Day 1 or 7 after tumor implantation. The effects of PAL on tissue phenylalanine and tyrosine levels are described in table 2. Free phenylalanine and tyrosine levels were only partially lowered in liver, muscle, kidney, or tumor cells of mice treated with 300, 600, or 1200 IU PAL/kg/day. A dose of 1200 IU PAL/kg/day did not seem to lower free phenylalanine or tyrosine in cells more than a dose of 600 IU/kg.

TABLE 1.—Effect of PAL on ascites tumors L5178Y and EARAD-1*

Tumor	Treatment	No. of mice in group	Average weight change (g)	Median survival time in days (range)
L5178Y	Tumor control	30	+0.4 (Day 6)	16 (13-18)
L5178Y	PAL	10	−0.5 (Day 6)	16 (13-18)
EARAD-1	Tumor control	5	+1.0 (Day 5)	15 (14-18)
EARAD-1	PAL	5	−3.3 (Day 5)	17 (16-19)†
EARAD-1	PAL + deficient diet	5	−5.9 (Day 5)	17 (13-19)†
EARAD-1	Deficient diet	5	−2.8 (Day 5)	17 (16-19)†

*BDF_1 mice were injected ip with 1×10^5 L5178Y cells and $B6AF_1$ mice with 4×10^5 EARAD-1 cells on Day 0. Mice received 3 daily ip injections of PAL (3×100 IU/kg) from Days 1 to 5. From Days 0 to 5, the experimental animals were fed either a complete synthetic diet or, where indicated, a synthetic diet deficient in phenylalanine and tyrosine.

†The significance of the increase in median survival time was $P > 0.05$.

TABLE 2.—Effect of PAL treatment on tumor and normal tissue phenylalanine (Phe) and tyrosine (Tyr) concentrations*

Enzyme dose (IU/kg/day)	Molar ratios of free phenylalanine and tyrosine (mols/mol of leucine)							
	EARAD-1 tumor		Liver		Kidney		Muscle	
	Tyr	Phe	Tyr	Phe	Tyr	Phe	Tyr	Phe
0 (Control)	1.4	0.47	0.63	0.32	0.84	0.33	1.8	0.55
	1.4	0.55	0.44	0.45	0.60	0.34	1.3	0.60
300 (2 × 150)	0.31	0.22	—	—	—	—	—	—
	0.23	0.24	—	—	—	—	—	—
600 (2 × 300)	0.14	0.09	0.16	0.12	0.36	0.17	0.19	0.14
	0.14	0.11	0.14	0.11	0.25	0.23	—	—
1200 (2 × 600)	0.16	0.12	0.16	0.11	0.22	0.19	0.15	0.12
	0.20	0.14	0.16	0.12	0.26	0.21	0.18	0.18

*There were 2 $B6AF_1$ mice per group. On Day 0 each mouse received 4×10^5 EARAD-1 cells sc. Mice received 2 daily ip injections of PAL on Days 7, 8, and 9 and 1 injection on Day 10. Tissue samples for amino acid analyses were obtained 3-4 hrs after the last enzyme injection was administered.

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Phase I-II Trial of VM-26 (NSC-122819) in the Treatment of Children With Late-Stage Leukemia[1,2,3]

Brief Reports and Preliminary Communications

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VM-26, a semisynthetic derivative of podophyllotoxin (1), has been shown to be effective against several animal leukemias and solid tumors including Walker 256 carcinosarcoma and L1210 mouse leukemia. Responses have been seen in human lymphoma, bladder cancer, and brain tumors (2-5). These early studies have been recently reviewed by Goldsmith et al (6). The mechanism of action appears to be similar to that of the vinca alkaloids, but patients with Hodgkin's disease who were resistant to MOPP[5] responded to VM-26 (7,8). Because of the animal studies and the reported effect on human lymphoma, a phase I-II trial in children with late-stage leukemia was instituted.

PATIENTS AND METHODS

Thirteen children with advanced-stage acute leukemia were treated with VM-26 from February 1971 through March 1973. Each had previously had one or more courses of treatment with vincristine, prednisone, methotrexate, 6-mercaptopurine, and cytosine arabinoside; 12 had had prior treatment with L-asparaginase and cyclophosphamide; nine had had prior daunorubicin; and five had had one or more other agents. Twelve had acute lymphoblastic or stem cell leukemia and one patient had acute histiocytic leukemia. The mean age at diagnosis was 6 years and 10 months; the median age was 5 years with a range of 2-13 years. The median time from diagnosis to treatment with VM-26 was 20 months. All patients entered in the study were in obvious clinical relapse and had had no changes in therapy for the preceding 2 weeks. Bone marrow examinations were not performed on patients with circulating leukemic blast cells. An adequate trial was defined as 2 weeks of treatment with progressive disease and 3 weeks with no response.

The children were entered in the study in groups of four. Each group began therapy at a higher dose than the initial dose of the preceding group. Each group had weekly escalation of the dose during the therapy. The initial doses ranged from 1 to 4 mg/kg twice weekly. The drug was diluted in a 250-ml infusion of 5% dextrose and given iv over a 30-minute period. The patients' status during therapy was evaluated by physical examination peripheral blood count, and bone marrow examination.

RESULTS

One of the 13 patients who received only two doses of VM-26 prior to death is excluded as having had an inadequate trial. Three patients received four doses of VM-26 and died of infection or hemorrhage with no evidence of response; these are included in the analysis as having had an adequate trial of therapy. The remaining nine patients received between six and 13 doses at a total dose range of 10-47 mg/kg per patient.

No objective signs of improvement were seen. No bone marrow examination showed a significant decrease in the percentage of blast cells or a decrease in cellularity. Peripheral counts in ten of 12 patients showed a static or increasing percentage of blast cells. Two patients showed a decrease in both the peripheral white blood cell (wbc) count and in the percentage of blast cells; however, both patients died within 2 weeks of completion of therapy—one from sepsis and leukemia and the other from a presumed intracerebral hemorrhage. Ten patients entered the study with leukocyte counts of 4000 cells/mm^3 or less and only one started with a wbc count in excess of 20,000 cells/mm^3. One child had a marked decrease in bone pain after the second dose at 2 mg/kg and the improvement continued. There were no other subjective signs of improvement.

Toxicity was minimal at all doses. On entering the study, all the children had thrombocytopenia and neutropenia as a result of leukemic infiltration. Twelve of the 13 children received red blood

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[3]VM-26: 4'-demethylepipodophyllotoxin, 9-(4,6-*O*-2-thenylidene-β-D-glucopyranoside).

[4]The Children's Hospital of Philadelphia, Pa.

[5]MOPP = mechlorethamine, vincristine, prednisone, and procarbazine.

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cell transfusions and eight received platelet transfusions. Seven of 13 had significant infections while receiving VM-26. Occasional mild nausea, which responded readily to antiemetics, was experienced by most patients. Alopecia, which has been previously reported, was difficult to evaluate since most of the children had significant alopecia from other agents received prior to entering this study. One patient had mild hypotension during infusion of VM-26 but no drop in blood pressure was observed with subsequent doses being administered at a slower rate, ie, longer than 30 minutes.

DISCUSSION

VM-26 was given to 13 children with end-stage acute leukemia. The starting dose of 1 mg/kg twice weekly for the first group of four patients was increased in each subsequent group of patients and the dose was escalated weekly within each group. In three patients the total dose was < 10 mg/kg which may have been inadequate treatment.

No objective responses were seen, although in two children there was a decrease in both the elevated wbc count and in the percentage of blast cells. One of those two children received considerable relief from bone pain.

The lack of response could be due to the far-advanced condition of the patients selected, their resistance to multiple agents including the vinca alkaloids, or an incorrect time or dose schedule. Although patients with end-stage leukemia are not ideal subjects on whom to test new antitumor agents, seven in this study lived > 4 weeks which should be sufficient time to achieve a remission. Other agents have demonstrated their effectiveness in patients such as these.

It is difficult to separate the drug effect from that of the disease when measuring toxicity in patients with active leukemia. Evidence of marrow suppression was noted to varying degrees in all 20 brain tumor patients treated by Sklansky et al (5). However, in only three patients was it clinically significant, each of whom had earlier shown severe marrow toxicity to nitrosourea therapy. Two of Sklansky's patients exhibited transient, mild hypotension when VM-26 was administered rapidly, ie, < 20 minutes, but not with more prolonged administration periods. The dose range for patients in that study was 3-4 mg/kg in weekly doses. This dose is similar to that given to five of the patients in our study, and in none of these patients was therapy stopped or reduced because of marrow toxicity. The majority of patients (ten of 13) had the same or an increased number of peripheral blast cells at the end of their course of treatment. This suggests that insufficient drug dosage was given to affect either the leukemia or the normal leukocytes. Each of the schedules reported by Mathé et al (2), which included daily, 5-day, and weekly administration, induced some hematologic toxicity in 17 of 55 patients. The median dose was 10-15 mg/kg, the same range as in this present study. Future trials should probably start with a dose of 4 mg/kg twice weekly and increase by 2 mg/kg each week until significant toxicity and/or objective response is seen. If possible, children with a longer expectation of survival should be selected to allow for a better evaluation.

The lack of response is disappointing in view of the animal data and reports of an effect in human lymphoma. A similar patient response to acute lymphoblastic leukemia was reported following an EORTC trial (9). The analog of VM-26, VP-16-213,[6] has induced complete remission in five of 16 patients with monocytic leukemia but no responses in six patients with acute lymphoblastic leukemia (2).

The results of the present study suggest that VM-26 is not a useful agent in the treatment of acute lymphoblastic leukemia of childhood. Further studies may be indicated, at higher dose levels, in lymphoma, acute myelocytic leukemia, and brain tumors.

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[6]VP-16-213: NSC-141540; 4'-demethylepipodophyllotoxin 9-(4,6-*O*-ethylidene-β-D-glucopyranoside).

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Preliminary Report on the Use of CCNU (NSC-79037), Adriamycin (NSC-123127), and Hexamethylmelamine (NSC-13875) in Carcinoma of the Lung[1,2,3,4]

Brief Reports and Preliminary Communications

W. L. Wilson,[5,*] N. C. Andrews,[6] R. W. Frelick,[7] T. F. Nealon,[8] R. L. Bick,[5] and T. Adams[5]

In the past, single-agent therapy has been used for carcinoma of the lung. The Central Oncology Group (1) has reported that hexamethylmelamine is effective in squamous cell carcinomas and dibromodulcitol is effective in adenocarcinomas. Other new single agents shown to induce a significant regression rate include bleomycin (2), CCNU (3), and adriamycin (4). Mosely et al (5) have reported on a regimen of CCNU, bleomycin, vinblastine, and adriamycin which has shown some success in squamous cell carcinomas, but these included only a few squamous cell carcinomas of the lung.

The objective of the Central Oncology Group was to do a pilot study utilizing CCNU, adriamycin, and hexamethylmelamine for squamous cell carcinomas of the lung. All of these agents have been shown to be effective in squamous cell carcinomas. CCNU has the additional advantages of its lipid solubility and its ability to cross the so-called blood-brain barrier. This, we felt, was important because of the high incidence of CNS metastases in any type of lung carcinoma.

Initial dosages were 70 mg/m^2 orally on Day 1 for CCNU, 15 mg/m^2 on Day 1 and then weekly by iv push for adriamycin, and 6 mg/kg orally in three or four divided doses for hexamethylmelamine starting on Day 1 and continuing until hematologic toxicity occurred. Very little hematologic toxicity was observed however, and the dose of CCNU was increased to 100 mg/m^2. The weekly adriamycin and daily hexamethylmelamine doses were discontinued if the white blood cell (wbc) count was < 3000 cells/mm^3 or the platelet count was < 75,000 cells/mm^3. After recovery from myelosuppression, the patient received a second cycle of the drug combination. If the wbc count dropped to < 1500 cells/mm^3, the dose of CCNU was reduced by 20 mg/m^2.

Eight patients received the 100-mg/m^2 dose of CCNU. All patients had metastatic disease with histologic proof of malignancy at the start of therapy. Three of the patients had received previous irradiation and had had disease recurrence. The others had metastatic disease at the time of diagnosis, and chemotherapy had been the primary treatment.

Of the seven patients who had multiple courses of therapy, two required reduction of the dose of CCNU because of prolonged leukopenia. Reduction occurred with the third course in one patient and with the fifth course in the other patient. The duration of each course varied considerably even for individual patients. The interval from the dose of CCNU to a wbc level of < 3000 cells/mm^3 varied from 4 to 7 weeks, at which time the adriamycin and hexamethylmelamine were discontinued. The intervals between doses of CCNU varied from 6 to 13 weeks (the latter was with the fourth dose of CCNU) before the wbc count rose to > 4500 cells/mm^3, at which time the next dose of CCNU was given and the adriamycin and hexamethylmelamine were resumed.

Adriamycin and hexamethylmelamine were given for approximately the first two thirds of each course of therapy and were resumed when the next dose of CCNU was administered. The neurotoxicity occasionally seen with hexamethylmelamine was not noted in any of these patients, probably because the drug was given intermittently. Stomatitis and cardiotoxicity associated with the administration of adriamycin were also not observed. This is probably also related to the intermittent low-dose schedule used. The largest total

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[2]Supported by Public Health Service grant CA-12295 from the National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare.

[3]This study was conducted by the Central Oncology Group.

[4]CCNU: CAS reg. No. 13010-47-4; urea, 1-(2-chloroethyl)-3-cyclohexyl-1-nitroso-.

Adriamycin: CAS reg. No. 23214-92-8.

Hexamethylmelamine: CAS reg. No. 645-05-6.

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dose of adriamycin given to any patient was 400 mg/m^2. Following this protocol it would probably take > 18 months to exceed the accepted total dose of adriamycin, which is considerably longer than the usual survival for patients with metastatic carcinoma of the lung. If the dose of adriamycin reaches a level of 550 mg/m^2, our recommendation is that the drug be dropped from the regimen since at this time there does not appear to be any reliable method of predicting the cardiotoxicity. A total of six of the eight patients showed a response to this regimen.

RESPONSE CRITERIA

Our criterion for a partial response is a 50% decrease in the sum of the products of the measurable disease of two measurements taken > 2 weeks apart. However, the most important factor in carcinoma of the lung is still survival, which is why our current regimen appears to be promising for further study. Two of the patients with epidermoid carcinoma showed a response in pulmonary and cerebral metastases. One patient with large cell carcinoma showed a response of her pulmonary metastases as well as a response in a large solitary bone lesion in the right ilium. She is also a long-term survivor.

Table 1 shows the survival and patient characteristics for those who received previous chemotherapy.

A significant response rate has been observed in this study, particularly in epidermoid carcinomas,

TABLE 1.—Patient characteristics and survival for those who received previous chemotherapy

Patient No.	Sex/age	Cell type	Dose of CCNU (mg/m^2)	Toxicity* Wbc level	Toxicity* Platelet level	Evaluable disease	Response	Survival (mos)	Comment
1	F/39	Adenocarcinoma	100	0	0	Lung	None	6	2+ nausea with hexamethylmelamine
2	M/69	Adenocarcinoma	100	3+	3+	Lung, bone	None	1.5	Dead; previous therapy = irradiation, methotrexate, 5-FU, cyclophosphamide
3	M/65	Undifferentiated	100	3+	3+	Lung	Partial	15	Alive
4	M/51	Anaplastic	100	3+	3+	Lung, subra nodules	Partial	9.5	Dead
5	F/71	Large cell	100	3+	3+	Lung, bone	Partial, lung and bone	15	Alive
6	M/55	Epidermoid	100	3+	3+	Lung	Partial	15	Died of liver involvement; had CNS disease at time of death; had previously received irradiation
7	M/60	Squamous	100	4+	4+	Lung, bone	Partial, lung; improved CNS function	4	Patient was an alcoholic and died suddenly with pneumonia; had previously received irradiation
8	M/68	Squamous	100	3+	4+	Lung, brain	Partial, lung and brain	5	Alive; brain lesions no longer visible on scan

*Wbc (cells/mm^3): 1= 3000-4000; 2 = 2000-3000; 3 = 1000-2000; 4 =< 1000. Platelets (cells/mm^3): 1 = 125,000-100,000; 2 = 75,000-100,000; 3 = 50,000-75,000; 4 =< 50,000.

but the number of patients is too small and the staging too inadequate to draw any real conclusions as to the efficacy of this drug combination using CCNU at a dose of 100 mg/m^2. However, the fact that six of eight patients responded appears to warrant further study, particularly since two of the three patients with CNS metastases showed a response. Only one patient had an infectious complication, and he was an alcoholic who, while ill with pneumonia, was not seen by any of us. The toxicity was moderately severe but was warranted, considering the lethal nature of this disease. The treatment program has been incorporated into phase III protocols of the Central Oncology Group for stage II-III carcinomas of the lung.

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Response of Metastatic Malignant Carcinoid Tumor to Adriamycin (NSC-123127)[1,2,3]

Brief Reports and Preliminary Communications

Alan Solomon,* Takuo Sonoda, and Frances K. Patterson[4]

Metastatic malignant carcinoid tumors are highly resistant to radiation therapy and are generally resistant to chemotherapy (1,2). This report was prompted because of the extremely rapid regression, on three separate occasions, of an extensive intra-abdominal metastatic malignant carcinoid tumor of the small intestine which occurred in a patient treated with adriamycin given as part of a combination chemotherapeutic regimen which included cyclophosphamide and methotrexate, and on a fourth occasion when adriamycin was used alone.

CASE REPORT

A 42-year-old woman was hospitalized in September 1974 for evaluation of an iron-deficiency anemia associated with fecal blood loss, diffuse, cramping abdominal pain, diarrhea, anorexia, and a weight loss of 11 pounds. Extensive diagnostic studies were performed; most significant was the finding by splanchnic arteriography of a large mass in the region of the distal ileum and adjacent ascending colon. Multiple irregularities were apparent in the blood vessels within and draining the mass; the right renal collecting system was partially obscured and a mild hydronephrosis of the right kidney was present. A chest roentgenogram and hepatic and skeletal radioisotopic scans showed no evidence of metastatic disease, and no urinary 5-hydroxyindoleacetic acid was detected. An exploratory laparotomy revealed a large ulcerating mass in the terminal ileum extending into the retroperitoneal area with extensive metastases to lymph nodes at the root of the mesentery and base of the superior mesenteric artery. A portion of the tumor was resected and an ileotransverse colostomy was performed. Histologically, the tumor was diagnosed as a carcinoid and was judged to be highly malignant on the basis of striking mitotic activity and by the extensive invasion of surrounding tissue and lymph nodes. The patient's postoperative course was uneventful, and the abdominal pain and diarrhea subsided.

Because of the residual intra-abdominal tumor, a chemotherapy regimen was instituted which included cyclophosphamide (400 mg/m^2) and BCNU (75 mg/m^2) given iv every 4 weeks.[5] The patient received three courses of treatment; early in December 1974 symptoms of intestinal obstruction developed. A firm, tender abdominal mass, approximately 10 cm in diameter, was palpated in the periumbilical region. Gastrointestinal roentgenograms disclosed an irregular mass invading the duodenum with mucosal distortion, most likely representing metastatic disease, and an iv pyelogram showed persistence of the compression of the right midureter with moderate hydronephrosis. The serum alkaline phosphatase level was increased twofold above normal and a technetium 99m sulfur colloid scan showed multiple mass lesions within the liver (fig 1A). A gallium 67 citrate scan showed prominent localization of radionuclide in the right midabdomen (fig 2A). The chemotherapy regimen was changed to a combination of adriamycin (60 mg/m^2), cyclophosphamide (600 mg/m^2), and methotrexate (25 mg/m^2) given iv every 3 weeks.[6] The abdominal pain and mass diminished considerably within 2 weeks after the first course of treatment and subsided completely prior to the third course of chemotherapy. A repeat gallium 67 scan (fig 2B) showed only faint localization in the area of previous mass and no abnormalities were evident on gastrointestinal roentgenographic examination. Furthermore, there was no sign of renal obstruction and the serum alkaline phosphatase level was normal; considerable reduction in the size and number of lesions was noted in an isotopic liver scan performed in January 1975 (fig 1B). The patient remained asymptomatic and the gallium 67

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[2]Supported in part by Public Health Service grants CA-10056 and CA-13237 from the National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare.

[3]Adriamycin: CAS reg. No. 23214-92-8.

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[5]BCNU: NSC-409962; CAS reg. No. 154-93-8; urea, 1,3-bis(2-chloroethyl)-1-nitroso-.

Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,-3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate.

[6]Methotrexate: NSC-740; CAS reg. No. 59-05-2; glutamic acid, *N*-[p-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-.

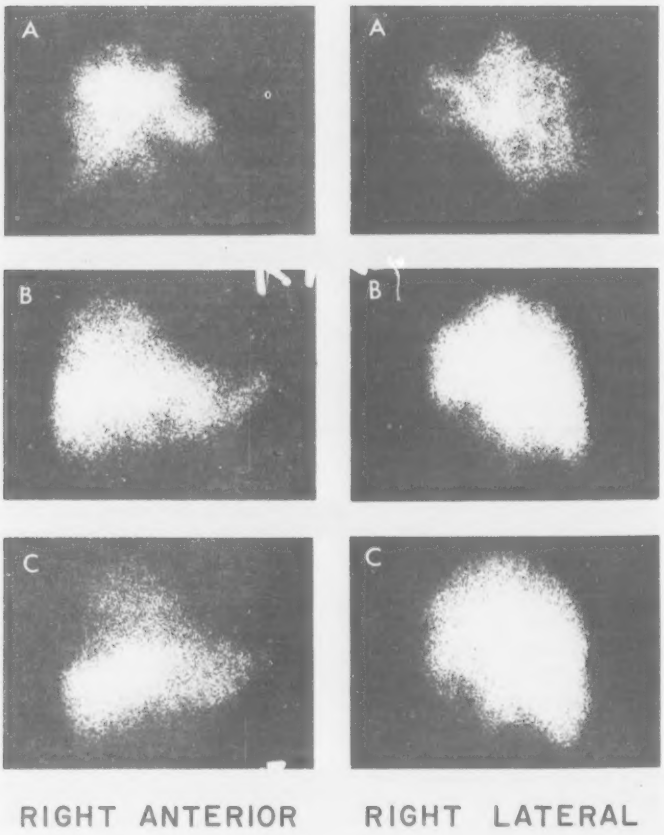

FIGURE 1.—Technetium 99m sulfur colloid liver scans. The multiple hepatic filling defects evident in the Dec 1974 scan (A) were decreased in the Jan 1975 scan (B) and were virtually undetectable in the June 1975 scan (C).

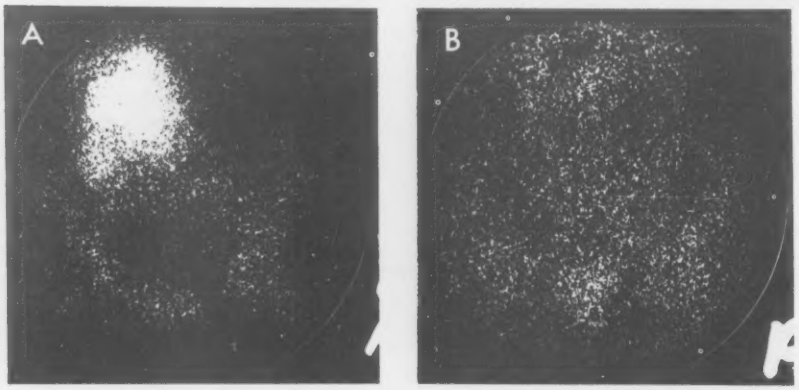

FIGURE 2.—Gallium 67 citrate scans. The area of abnormal radionuclide uptake in the right mid-abdominal region seen in the Dec 1974 scan (A) was not evident in the Jan 1975 scan (B).

and liver scans (fig 1C) were normal after completion of the sixth course of chemotherapy in April 1975. One course of cyclophosphamide and methotrexate was given in June 1975, and 4 weeks later the patient experienced abdominal pain and again an abdominal mass was palpable. Gastrointestinal roentgenograms disclosed a mass approximately 5 cm in diameter compressing the distal ileum. Because of the previous marked response achieved with adriamycin in combination with cyclophosphamide and methotrexate this regimen was again given, and within 1 week there was marked symptomatic improvement and rapid reduction in size of the abdominal mass; gastrointestinal roentgenograms taken 3 weeks later were normal. However, 11 weeks after the chemotherapy was given, a gallium 67 scan revealed slight uptake of radionuclide in the mid-abdominal region and 2 weeks later a large abdominal mass was palpable. The mass regressed after administration of adriamycin *alone*; however, the tumor was again palpable at 3 weeks and by 5 weeks it was considerably larger. Treatment with adriamycin, cyclophosphamide, and methotrexate again resulted in prompt tumor regression and no disease was evident on examination 5 weeks later. We plan to administer the three drugs in combination every 10-12 weeks until a cumulative dose of adriamycin (550 mg/m^2) is reached.

DISCUSSION

Therapy for metastatic malignant carcinoid tumors of the small intestine is limited primarily to control of pharmacologic symptoms (when present) by means of antihormonal therapy (1,2), surgical excision of the metastatic lesions (particularly partial hepatectomy for solitary hepatic metastasis [1]), and, if necessary, use of cytotoxic agents. A variety of such compounds, including cyclophosphamide, thioTEPA, mechlorethamine, 5-fluorouracil, melphalan, actinomycin D, BCNU, mitomycin C, and streptozotocin, have been employed singly with only limited success (1-5). The use of drugs in combination, eg, methotrexate and cyclophosphamide, has resulted in an improved remission rate compared to the use of either agent alone (2,3). Our patient received cyclophosphamide and BCNU postoperatively; however, within 3 months there was evidence of extensive hepatic and intra-abdominal metastases. Because of the documented effectiveness of adriamycin used either singly or in combination with other types of antineoplastic agents in a variety of solid tumors and certain hematologic malignancies (6), this drug was included as part of a combination chemotherapy protocol. Rapid regression of metastatic tumor was evident after the first course of treatment, and the entire course of this therapy given every 4 weeks for 6 months was well tolerated. Continued treatment with cyclophosphamide and methotrexate was *not* sufficient to prevent relapse. The reinstitution of adriamycin as part of the combination chemotherapeutic regimen at the time of the second relapse and its use singly at the time of the third relapse again resulted in rapid regression of tumor. However, only a partial and relatively brief response was achieved with adriamycin alone and subsequently the three-drug combination again resulted in the marked and sustained effect achieved on the two previous occasions. Although it would appear that adriamycin was primarily responsible for the observed tumor regression, a synergistic therapeutic effect was achieved with the additional combination of cyclophosphamide and methotrexate.

The use of radionuclide scans provided an additional objective measure of disease activity and of response. The localization of gallium 67 in Hodgkin's disease and other types of malignancies as well as in inflammatory lesions has been demonstrated (7,8). Heretofore, there has been no report of gallium 67 uptake by malignant carcinoid tumors, thus the localization of this radionuclide in the intra-abdominal tumor of our patient was of special interest. Recently, a positive scan with technetium 99m pertechnetate in a patient with a malignant carcinoid tumor of the ileum has been described (9). However, the general usefulness of gallium 67 or technetium 99m pertechnetate as scanning agents for carcinoid tumors, as well as the efficacy of adriamycin alone or the combination of adriamycin, cyclophosphamide, and methotrexate, must be substantiated by treatment of additional patients with metastatic malignant carcinoid tumors of the intestine.

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Study of Leo-1031 (NSC-134087) in Lymphocytic Lymphoma and Chronic Lymphocytic Leukemia[1,2,3]

Brief Reports and Preliminary Communications

J.H. Kaufman,* G.L. Hanjura, A. Mittelman, C.W. Aungst, and G.P. Murphy[4,5]

Combination chemotherapy with chlorambucil and prednisone in chronic lymphocytic leukemia (CLL) produced an 87% response rate compared to a 50% response rate with chlorambucil alone (1).[6] Similar results have been observed in lymphocytic lymphoma (LL) (2). Since this combination of an alkylating agent and a corticosteroid proved effective in these diseases, the AB Leo Company combined a series of corticosteroids with alkylating agents to form single molecule esters. This led to the development of Leo-1031.

Leo-1031 has been found effective in a variety of animal tumors (3) including L1210 leukemia and Walker 256 carcinosarcoma. Since corticosteroids have already been shown to have selective uptake in both CLL and LL cells (4), it was postulated that the corticosteroid would act as a carrier for the alkylating agent to cross cell membranes. Past work seemed to indicate that the lipophilic ester of alkylating agents might remain intact for a sufficient length of time to carry the alkylating agent into previous sanctuary areas (5).

MATERIALS AND METHODS

Thirteen ambulatory patients with histologically confirmed stage III and IV LL and eight patients with CLL were treated with Leo-1031 at an initial daily dose of 8 mg/day for CLL and 24 mg/day for LL. Dosage was modified by hematologic toxicity. The doses were escalated by 4 mg/day every 6 weeks when the neutrophil count was 2500 cells/mm^3 and the platelet count was 100,000 cells/mm^3; 50% of the dosage was given when the neutrophil count was 1500 cells/mm^3 and the platelet count was 50,000 cells/mm^3, and no drug was given when the blood cell counts fell below these levels. All patients had measurable disease with 16 patients having relapsed after prior therapy (corticosteroids, nine patients; chlorambucil, seven patients). Three patients had relapsed during treatment; the remainder were in unmaintained remission.

Remission criteria for CLL and LL have been previously published (1,6), with a CR defined as the absence of all objective evidence of tumor (including anemia and thrombocytopenia) and a PR defined as a 50%-90% decrease in the cross-sectional area of all measurable tumors.

RESULTS

In the eight patients with CLL (table 1) there were two PRs, four nonresponders, and two nonevaluable patients. The two nonevaluable patients had treatment discontinued for allergic dermatitis (one patient) and an invalid condition (one patient). Among the 13 patients with LL there were three CRs, seven PRs, two failures, and one nonevaluable patient (drug was discontinued at patient's request), giving ten responders (77%) (table 1). The median duration of treatment was 100 days with a range of 49-360+ days. Two of the three patients who had relapsed during alkylating agent and prednisone therapy responded to Leo-1031.

Among the eight patients with a nodular cell type, there were two CRs and five PRs. Among the five patients with a diffuse cell type, there were two PRs and one CR.

TOXICITY

Leukopenia requiring dosage reduction occurred in ten patients. Thrombocytopenia without clinical bleeding required dose reduction in three patients and discontinuation in one. A median maintenance dose of 10 mg for CLL and 16 mg for LL was given. No dosage escalation was possible in patients with LL, but escalations of up to 24 mg/day were occasionally possible in those with CLL.

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[2]Supported by Public Health Service grant RR-262 from the General Clinical Research Centers Program, Division of Research Resources, National Institutes of Health, Department of Health, Education, and Welfare.

[3]Leo-1031: CAS reg. No. 29069-24-7; pregna-1,4-diene-3,20-dione, 11β,17,21-trihydroxy-, 21-[4-[p-[bis(2-chloroethyl)amino]phenyl]butyrate].

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[5]We wish to extend our thanks to Leon Stutzman, MD, and Miss Nancy Riffel for their assistance in preparing the manuscript.

[6]Chlorambucil: NSC-3088; CAS reg. No. 305-03-3; butyric acid, 4-[p-[bis(2-chloroethyl)amino]phenyl]-.

Prednisone: NSC-10023; CAS reg. No. 53-03-2; pregna-1,4-diene-3,11,20-trione, 17,21-dihydroxy-.

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TABLE 1.—Response of lymphocytic lymphoma and chronic lymphocytic leukemia to Leo-1031

Patients	Response*	Duration of treatment (days)	Prior treatment (response)
Lymphocytic lymphoma			
1	CR	360+	Radiotherapy, vincristine (CR)
2	CR	300	Chlorambucil (CR)
3	CR	100	None
4	PR	330+	Radiotherapy (CR)
5	PR	110+	Radiotherapy, cyclophosphamide, prednisone, chlorambucil (CR)
6	PR	110+	MOPP (1 course) (NR)
7	PR	90+	Cyclophosphamide, prednisone, BCNU, vincristine (CR)
8	PR	85	Chlorambucil (CR)
9	PR	68	None
10	PR	49†	Chlorambucil, prednisone (CR)
11	NR	140	Vincristine, streptonigrin, cyclophosphamide, prednisone (NR)
12	NR	28	None
13	NE	21	Radiotherapy (CR)
Chronic lymphocytic leukemia			
1	PR	420+	Chlorambucil, prednisone (PR)
2	PR	440+	Chlorambucil, prednisone (CR)
3	NR	47	None
4	NR	63	Chlorambucil, prednisone (PR)
5	NR	130	None
6	NR	270	Total nodal radiation (CR)
7	NE	4	Chlorambucil, prednisone (NR)
8	NE	7	None

*CR = complete remission; PR = partial remission; NR = no response; NE = nonevaluable.
†Died in continued PR.

The drug was discontinued because of nonhematologic toxicity in four patients: nausea and vomiting in one patient and skin reactions in three (one patient had prior chlorambucil allergy). No steroid effects beyond moderate elevation of blood glucose were noted.

DISCUSSION

The response rate of 77% in LL is comparable to previously reported figures of 75% with Leo-1031 (7). The 25% response in CLL is considerably lower than that reported in prior studies and may be a result of the strict criteria of response.

The response of seven of eight nodular lymphomas compared to three of five diffuse lymphomas is in accordance with previously described results of better responses with nodular cell types (8). The median dosage of 10 mg/day in CLL and 16 mg/day in LL was far below the previously reported dosage of 40 mg/day in LL (7), but would explain the lack of Cushingoid side effects in this study. Steroid toxicity with Leo-1031 has been reported by others (7). The higher dosage given in Sweden may be a result of the smaller numbers of patients with prior chemotherapy (four out of 20) and of the definition of leukopenia as 2000 cells/mm^3 (6) as compared to 4000 cells/mm^3 in this study. Allergic skin reactions to Leo-1031 were more frequent in our study (three of 21 patients) compared to chlorambucil alone (two of 126 patients) (6), and this effect has not been previously described with Leo-1031 (6,9).

The present study indicates that Leo-1031 may be a safe, long-term oral treatment for patients with LL and CLL. The response of patients who had previously relapsed during alkylating agent and corticosteroid therapy suggests a lack of cross resistance with Leo-1031.

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Phase I Study of Weekly Administration of Anhydro-ara-5-fluorocytidine (NSC-166641)[1,2]

Brief Reports and Preliminary Communications

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Anhydro-ara-5-fluorocytidine (AAFC) has been described as a "double-barreled" antitumor compound that has given rise to two active metabolites, 1-β-D-arabinofuranosyl-5-fluorocytosine, an inhibitor of DNA polymerase, and 1-β-arabinofuranosyl-5-fluorouracil, which, like 5-fluorouracil, inhibits thymidylate synthetase (1-5).

Among different animal species, the dog is the most sensitive to AAFC (6-8). When daily iv injections were given over a 2-week period, five times/week, 10 mg/kg/day was well tolerated but 20 mg/kg/day was lethal. The toxic manifestations in animals consisted of bone marrow depression, intestinal lesions, and some cardiopulmonary damage.

Kreis et al (4) and Tan et al (9) at the Memorial Sloan-Kettering Cancer Center in New York have already presented the results of a phase I study. AAFC was given daily or every 12 hours for 3 days or more by a direct iv route. With this schedule, the proposed dosage for further administration, particularly in acute leukemia, was 20 mg/kg of body weight for 5 days or more in adults, or 15 mg/kg every 12 hours in children.

Our phase I study investigated the tolerance and toxic effects of AAFC when it was given weekly to adult patients with solid tumors.

PATIENTS AND METHODS

All patients who received AAFC had advanced malignant tumors that were confirmed by histology or cytology. These patients had an estimated survival time of at least 2 months and were conscious and communicative so that neurologic or emotional effects of the drug could be readily evaluated.

AAFC was given in weekly doses to consecutive groups of patients, with the first group receiving the basic dose of 10 mg/kg/week. Each successive group received a higher dose than the preceding group, after the latter had completed 3 weeks of the program. Each patient received a maximum of six identical successive weekly doses. The dose was not modified for each patient, but the treatment was discontinued when necessary. Each group included three patients until toxic effects were observed and five or more patients for higher dose levels. The dose escalation was determined by a modification of the Fibonacci program which is commonly used in phase I studies.

Administration of AAFC was by rapid iv injection for the lower dose groups until the appearance of toxic signs; at this point, the number of patients in each group was doubled with half receiving rapid iv injection and half receiving the same dose by 2-hour iv drip.

AAFC is available in 10-ml ampules of dry, lyophilized substance, each containing 200 or 500 mg of active product. Solutions were prepared by the addition of sterile 5% glucose. Five or 10 ml was added for rapid injections and 500 ml was added for use as a 2-hour iv drip.

RESULTS

Rapid Iv Injections (Table 1)

Twenty-four patients were treated by rapid iv injection. The mean age was 65 years. Nine patients had epidermoid head and neck carcinoma, 12 patients had adenocarcinoma (four, prostate; three, pancreas; two, breast; one, rectum; one, lung; and one, ovary), and three patients had miscellaneous tumors. Three of these 24 patients received the lowest dose of 10 mg/kg/week. One treatment was interrupted after 3 weeks because of transient thrombocytopenia. For the other patients, no toxic effects were observed. Seven patients received 20 mg/kg/week. At this level, mild hematologic toxic effects were present, as well as tolerable nausea and vomiting. No treatment was interrupted for toxic effects, but five patients died

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[2]Anhydro-ara-5-fluorocytidine: CAS reg. No. 40505-45-1; *O*[2],2′-cyclocytidine, 5-fluoro-, monohydrochloride.

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before the sixth week because of tumor progression. Nine patients received 33 mg/kg/week. Treatment was discontinued in four of these patients before the sixth week because of leukopenia (two patients), profound asthenia (one patient), and cutaneous rash (one patient). Digestive toxic effects were more pronounced for the 33-mg/kg/week dose than for the 20-mg/kg/week dose, but no dose modification was required. Finally, five patients received 50 mg/kg/week. In all instances, the treatment was discontinued after the second injection because of leukopenia or thrombocytopenia, and asthenia.

Iv Infusion (Table 1)

Fourteen patients were treated by iv infusion over a 2-hour period. The mean age was 58 years. This group had ten patients with epidermoid cancer, two with adenocarcinoma, one with soft tissue sarcoma, and one with lymphoma. Four patients received 20 mg/kg/week. In two of these patients, the treatment was interrupted after 4 and 5 weeks because of thrombocytopenia or leukopenia. Digestive tolerance was acceptable. Nine patients received 33 mg/kg/week. In all but two of the nine patients the treatment had to be interrupted for hematologic toxic reactions (after 1 week in two patients, 2 weeks in two patients, 3 weeks in two patients, and 4 weeks in one patient). One patient died after a 3-week treatment because of agranulocytosis and sepsis. The incidence of nausea and vomiting was greater for the 2-hour infusion than for the same dosage given by rapid iv injection. One patient received one infusion of 50 mg/kg and experienced agranulocytosis with pneumonia from which he recovered.

CONCLUSIONS

The phase I study conducted by Tan et al (9) at the Memorial Sloan-Kettering Cancer Center has shown that AAFC when given at 20 mg/kg and administered for 5 consecutive days once every 3-4 weeks is tolerable and provokes myelosuppression which, although transient, may be quite important. We have tried to define the tolerable dose on a weekly injection basis, as this could be more appropriate for the treatment of outpatients with solid tumors. Taking into consideration the level of toxicity manageable for an ambulatory treatment, the weekly dose for further phase II studies seems to be in the vicinity of 33 mg/kg for adult patients. In all patients, myelosuppression was the dose-limiting factor. Nausea and vomiting was frequent but was not of major importance. Comparing rapid injection and 2-hour infusion, the former seemed to be better tolerated. Of 38 patients treated, two died from complications due to agranulocytosis. Although the purpose of this study was not to evaluate therapeutic results, we have nevertheless observed three objective partial remissions: two in epidermoid carcinoma and one in an adenocarcinoma of the pancreas.

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Table 1.—Results of phase I study of patients treated with rapid iv injections or 2-hr infusions of AAFC in weekly doses

No. of patients	Dose (mg/kg/wk)	Mean duration of treatment (wks)	Mean degree of toxicity*			
			Hematologic	Digestive	Cardiac	Other
			Rapid iv injections			
3	10	5	1	0.6	0	0
7	20	3.9	1.1	0.9	0	0.3
9	33	4.2	1.6	1.4	0	0
5	50	1.8	2	1.8	0.8	0
			2-Hr infusions			
4	20	4.3	1.3	1.3	0	0.3
9	33	3.1	2.3	1.5	0.4	0.4
1	50	1	3	2	2	1

*Degree of toxicity—0 = normal; 1 = mild; 2 = severe; 3 = life threatening.

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Phase II Study of Methyl-CCNU (NSC-95441) in Advanced Gastrointestinal Cancer[1,2,3]

P. F. Engstrom,* R. B. Catalano, and R. H. Creech[4]

In preliminary trials (1-3) the nitrosourea compounds have shown activity in patients with gastrointestinal adenocarcinoma. Methyl-CCNU has a definite advantage in that it can be administered orally on an outpatient basis. The purpose of this study was to evaluate the effectiveness and toxicity of methyl-CCNU in a series of patients with advanced gastrointestinal adenocarcinoma.

METHODS

Fifty-seven consecutive patients with biopsy-proven disseminated gastrointestinal cancer and measurable indicators of recurrent or metastatic disease were entered in this phase II study between September 1973 and January 1975. Forty men and 17 women with an age range of 32-80 years (mean age, 60 years) were included in the study. Although all patients had received prior chemotherapy or radiotherapy for their disease, their life expectancy was estimated to be > 2 months at the start of methyl-CCNU.

Before the start of methyl-CCNU therapy, patients were evaluated for extent of disease with the following tests: complete physical examination, blood cell count, tests of liver and renal function, chest roentgenogram, and liver scan. When specific symptoms indicated, a radiologic examination of the gastrointestinal tract, brain scan, electroencephalogram, bone scan and survey, and bone marrow biopsy were performed.

A patient was considered eligible for the study if the following requirements were fulfilled: no radiation therapy or chemotherapy within the previous 4 weeks; a white blood cell (wbc) count > 4000 cells/mm^3 and a platelet count > 100,000 cells/mm^3; and no evidence of severe impairment of renal or liver function (blood urea nitrogen, serum glutamic oxaloacetic transaminase, lactic dehydrogenase, and bilirubin must have been within 1½ times the normal values for the laboratory). Each patient gave his informed written consent before methyl-CCNU was administered.

All patients received methyl-CCNU orally as a single dose of 200 mg/m^2 of body surface area on Day 1 after a 2-hour fasting period. Courses were repeated every 49 days until evidence of disease progression occurred. Complete blood cell counts and biochemical determinations were repeated between Days 21 and 28 and on Day 49 of each course of therapy. The dose of each subsequent course was based on the nadir blood cell count produced at the previous dose level, and was modified according to table 1. If there was evidence of delayed myelosuppression on Day 49, the next course of therapy was delayed until recovery was evident (wbc count > 3000 cells/mm^3; platelet count > 100,000 cells/mm^3). At that time, the therapy was reinstituted at the dose appropriate for the nadir produced.

Opaque gelatin capsules containing 10, 50, or 100 mg of methyl-CCNU were provided for this study.

The following tumor response criteria (based on the product of the greatest perpendicular diameters) were used in this study: (*a*) *complete response*—disappearance of all malignant lesions for at least 2 months; (*b*) *partial response*—50% or greater shrinkage of tumor for at least 2 months; (*c*) *stable disease*—no increase but < 50% shrinkage of measurable tumor plus subjective improvement for at least 2 months; and (*d*) *progression*—> 25% increase in tumor during the treatment period. Response duration and survival were measured from the initiation of methyl-CCNU therapy.

RESULTS

Forty-seven of the 57 patients entered in this study completed at least 7 weeks of followup evaluation which constituted, for our purposes, the minimum adequate trial to determine response. The

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[3]Methyl-CCNU: CAS reg. No. 33073-59-5; urea, 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitroso-. Supplied by Cancer Therapy Evaluation Program, Division of Cancer Treatment, NCI.

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TABLE 1.—Dosage modification based on nadir blood cell count

Methyl-CCNU dosage (%)	Nadir count on Days 21-28	
	Wbc	Platelets
100	≥ 3000	≥ 75,000
75	2000-2999	50,000-74,999
50	>2000	< 50,000

ten unevaluable patients included six who died of progressive metastatic disease within 1 month of the start of treatment, three who were lost to followup, and one who received radiotherapy to the only site of measurable disease.

Table 2 summarizes the responses to methyl-CCNU therapy by disease site. Table 3 lists the pertinent data on the responders in this study.

Although no patient with colorectal carcinoma met the criteria for a complete or partial response, five of 26 patients experienced > 25% but < 50% antitumor activity (stable disease). Three other patients showed no progression in tumor parameters for 2 or more months. These eight patients had a mean duration of tumor stabilization of 3.5 months, with a mean survival of 7.5 months. The mean survival for the patients with progressive colorectal carcinoma was < 4 months.

The only complete response occurred in a patient with metastatic gastric carcinoma. He had a 6-month complete regression of a measurable biopsy-proved axillary node and a complete radiographic, endoscopic, and histologic regression of his unresected gastroesophageal adenocarcinoma. The methyl-CCNU was continued until the patient died of recurrent esophageal obstruction, 13 months after the start of therapy. A second gastric carcinoma patient had a partial regression of metastatic neck nodes for 8 months. Three other gastric carcinoma patients showed stabilization of their metastatic disease in the liver or the epigastric area for 3, 4, and 6 months respectively.

Measurable antitumor activity was noted in two patients with metastatic pancreatic carcinoma. The remaining 32 patients including two with biliary tract carcinoma showed no significant antitumor effect with methyl-CCNU therapy. The mean survival duration in the patients with progressive disease was < 3 months from initiation of the methyl-CCNU.

TOXICITY

Mild to moderate gastrointestinal upset occurred in 23 of 47 patients treated with methyl-CCNU. Many patients experienced nausea on the day of drug ingestion, but this could be ameliorated with chlorpromazine administration on the first 2 days of treatment. No patient refused or stopped therapy because of adverse gastrointestinal side effects.

Five patients experienced significant hematologic toxicity during the first course of methyl-CCNU therapy. Three patients who had extensive liver metastases but near normal liver function studies developed severe pancytopenia with platelet counts < 20,000 cells/mm^3. One patient who received previous pelvic radiotherapy had his death hastened by gastrointestinal bleeding secondary to thrombocytopenia within 8 weeks of initiating the methyl-CCNU chemotherapy. Two other patients who had received prior chemotherapy and extensive pelvic radiotherapy developed platelet count nadirs of 29,000 and 40,000 cells/mm^3 respectively.

By modifying the drug dosages, 15 patients received three or more (maximum of seven) courses of methyl-CCNU. There was a nonpredictable tendency toward a cumulative hematologic toxicity, so that many patients received less than two thirds of their predicted methyl-CCNU dosage or required increased intervals of 9-10 weeks between treatment courses before resuming therapy.

DISCUSSION

In order to use methyl-CCNU, the oncologist must appreciate its unusual bone marrow effect. Moertel (3) has described a bimodal leukocyte toxicity with nadirs on Days 14 and 35 after a single

TABLE 2.—Response to methyl-CCNU therapy

Site of disease	Total No. of patients	No. of evaluable patients	No. of patients with—			
			Complete response	Partial response	Stable disease	Progressive disease
Colorectal	31	26	0	0	8	18
Gastric	13	11	1	1	3	6
Pancreatic	11	8	0	2	0	6
Biliary tract	2	2	0	0	0	2

TABLE 3.—Clinical data on methyl-CCNU responders with gastrointestinal adenocarcinoma

Patient No.	Age (yrs)	Tumor type	Measurable disease	Prior therapy*	Response		No. of courses of methyl-CCNU	Survival (mos)
					%	Duration (mos)		
1	57	Rectum	Pelvic mass	5-FU, TGdR	25	4	4	4
2	46	Colon	Abdominal mass	5-FU	25	3	2	3
3	54	Rectum	Abdominal mass	5-FU	30	5	4	15
4	67	Colon	Flank mass	5-FU, TGdR	40	2	2	9
5	70	Colon	Abdominal mass, liver	5-FU	25	3	3	7
6	64	Stomach	Primary axilla	CYC	100	6	7	13
7	71	Stomach	Supraclavicular node	5-FU	50	8	6	13
8	51	Pancreas	Abdominal mass, liver	5-FU	50	14	7	19
9	40	Pancreas	Liver	5-FU	60	10	6	11

*5-FU = 5-fluorouracil; TGdR = β-2'-deoxythioguanosine; CYC = cyclophosphamide.

dose of methyl-CCNU. In a phase I trial, Young et al (2) noted the nonpredictable cumulative marrow suppression especially when patients received >175 mg/m^2 of methyl-CCNU. In this phase II study, patients tolerated 200 mg/m^2 every 7 weeks unless they had received prior chemotherapy plus pelvic irradiation or unless they had extensive liver metastases. We recommend that patients in either of these categories be considered poor-risk patients and receive modified doses of methyl-CCNU (150 mg/m^2) in order to prevent undue first-course thrombocytopenia. Bone marrow toxicity can be attenuated during repeat-course therapy with a dose-modification schedule such as employed in this study. It might also be advantageous to administer methyl-CCNU on an every-10-week cycle rather than every 7 weeks, to lessen cumulative bone marrow suppression.

We obtained antitumor activity with methyl-CCNU in previously treated patients with gastric and pancreatic adenocarcinoma. Further controlled trials are necessary to determine if methyl-CCNU is the preferred agent for these tumors. Moertel (5) reports that methyl-CCNU plus 5-fluorouracil is more effective than either agent alone in the management of advanced gastric adenocarcinoma.

The response of colorectal adenocarcinoma to methyl-CCNU was disappointingly poor. It is possible that previously untreated patients with smaller tumor burdens could benefit from this agent since we demonstrated antitumor activity less than partial response in five of our patients. Baker et al (4), in a preliminary report, indicate that 5-fluorouracil plus methyl-CCNU is more effective than 5-fluorouracil alone in the initial treatment of patients with measurable colorectal carcinoma.

We are presently exploring the use of methyl-CCNU in combination with other active drugs in the treatment of gastrointestinal adenocarcinoma.

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A Pharmacokinetically Based Phase I-II Study of Single-Dose Actinomycin D (NSC-3053)[1]

Brief Reports and Preliminary Communications

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Actinomycin D was the first crystalline antibiotic isolated from *Streptomyces* in 1940 by Waxman and Woodruff (1). It was found to have antitumor activity against murine Sarcoma 180 in 1950 (2) and against human tumors in 1952 (3). Actinomycin D, like the anthracycline antibiotics, is known to bind to DNA and to be retained in the body for a prolonged period of time (4). Recent work in several animal systems has shown the drug to have a half-life of approximately 2 days (5). These data indicate that the usual clinical practice of dividing the actinomycin D dose over a 5-day period may not be necessary and suggested to us the use of an intermittent single-dose schedule.

MATERIALS AND METHODS

Thirty-three adult patients with disseminated malignant disease were entered in the study. Twenty-six had malignant melanoma, three had breast carcinoma, two had sarcoma, and one each had carcinoma of the colon and carcinoma of the testis. At the start of the study all had progressive disease refractory to one and usually several previous chemotherapeutic regimens. Patients were evaluated before therapy with regard to tumor measurements as well as metabolic and hematologic parameters. The latter were to be repeated twice weekly during each course of chemotherapy; however, in some patients these data could not be obtained because of severe patient debility before the start of chemotherapy and an inability to make the required visits to the local physician. The initial dose was 2.5 mg/m^2 to be repeated in 3 weeks if recovery from toxicity was complete. Twenty-three patients were treated with that dose and schedule. Because of the degree of toxicity and the interval for recovery required in these patients, the dose was decreased to 2 mg/m^2 and the interval between doses was prolonged to 4 weeks in an additional nine patients. One patient was treated with a dose of 1.25 mg/m^2 because of a bilirubin level of 1.5 mg/100 ml. The drug was administered over 15 minutes after dilution with 50 ml of 5% dextrose in water.

Complete remission was defined as disappearance of all clinical evidence of active tumor for a minimum of 4 weeks together with a disappearance of all tumor-related symptoms. Partial remission was defined as a $\geq$ 50% decrease in the sum of the products of the largest perpendicular diameters of all measured lesions lasting at least 4 weeks in the absence of significant increase in any one lesion or the appearance of new lesions. Stable disease was defined as a steady state of response less than partial remission of at least 4 weeks' duration or progression less than progressive disease of at least 8 weeks' duration. Progressive disease was defined as unequivocal increase of any measured lesion or the appearance of new lesions.

RESULTS

Response

Of the 26 patients with malignant melanoma, two died within 2 weeks of the start of therapy and two others had progressive disease limited to the central nervous system, a pharmacologic sanctuary from actinomycin D. Therefore, of the 22 patients who could be evaluated for response, one (5%) had complete remission lasting 2 months and one (5%) had partial remission lasting 1.5 months for an overall remission rate of 9%. In addition, six patients (27%) had stabilization of their previously advancing disease lasting 1-5 months (median, 2 mos) while 14 (64%) showed clearcut progressive disease. It is noteworthy that the one complete responder and the one stable patient were among the four evaluable patients with melanoma

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treated at a dose of 2 mg/m^2. One of the three patients with breast cancer showed a transient but dramatic improvement of skin and subcutaneous metastases; this patient had had progressive disease following a combination chemoimmunotherapy regimen that included adriamycin. One of the two sarcoma patients died within the first few days of chemotherapy and the other had progressive disease. One patient with testicular carcinoma had stable disease for 5 months before progression, and the one patient with carcinoma of the colon has had stabilization of disease for 2 months.

Toxicity

Nonhematologic toxicity from single-dose actinomycin D could be evaluated in 26 patients; 19 were treated at a dose of 2.5 mg/m^2 and seven were treated at a dose of 2 mg/m^2 (table 1). Nausea and vomiting was present in at least 15 of the 19 patients, and although not mentioned, was not noted to be absent in the other four. In three patients, drug-related vomiting was present for > 3 days, requiring hospitalization in two patients. In two additional patients profound nausea and vomiting was considered to be disease related due to intestinal obstruction in one patient and increased intracranial pressure in another. Rash was present in seven of 19 patients (37%), was severe in five (26%), and was directly related to radiation or sun exposure in three. In contrast, vomiting was noted in only four of the seven patients evaluated at the 2-mg/m^2 dose, and the presence of mild nausea only was noted in an additional two. Mucositis occurred in two patients (29%), and rash was not observed at this dose level. Cardiotoxicity was not specifically evaluated, but was not evident during the rather brief period of observation. Hepatotoxicity was not noted.

TABLE 1.—Nonhematologic toxicity of single-dose actinomycin D

Toxic effect	No. of patients (%) treated at dose level of— 2.5 mg/m^2 (19 patients)*	2.0 mg/m^2 (7 patients)*
Vomiting	15(79)	4(57)
> 3 days	3(16)†	0(0)
Mild nausea only	0(0)	2(29)
Diarrhea	2(11)	2(29)
Dysphagia	2(11)	0(0)
Mucositis	9(47)	2(29)
Severe	2(11)	0(0)
Rash	7(37)	0(0)
Severe	5(26)	
Exacerbated by irradiation	3(16)	
Alopecia	2(11)	0(0)

*Total No. of evaluable patients.

†Two additional patients had prolonged vomiting related to their disease.

Hematologic toxicity was evaluated only when blood counts were recorded at least weekly. Thirteen patients were evaluated at a dose of 2.5 mg/m^2 and six at a dose of 2 mg/m^2 (table 2). The primary hematologic toxic effect was granulocytopenia. The median lowest granulocyte count occurred 16 days after treatment at either dose level and was 1.0 and 1.25 × 10^3 cells/mm^3 at the 2.5- and 2.0-mg/m^2 dose levels respectively. Nonetheless, granulocytopenia was transient with complete hematologic recovery by Day 23. Thrombocytopenia was unusual but did occur. When present, it tended to precede neutropenia with the lowest platelet count recorded on median Day 12-13. No patient required hospitalization for infectious or hemorrhagic complications of myelosuppression although one patient was hospitalized for pneumonia with normal blood counts. There was no evidence of cumulative myelosuppressive toxicity in eight patients started at a dose of 2.5 mg/m^2 although dose reductions were necessary in

TABLE 2.—Hematologic toxicity of single-dose actinomycin D*

Dose (mg/m^2)	No. of evaluable patients	Lowest recorded wbc count (cells × 10^3/mm^3)	Lowest recorded absolute granulocyte count (cells × 10^3/mm^3)	Day of lowest recorded absolute granulocyte count	Lowest recorded platelet count (cells × 10^3/mm^3)	Day of lowest recorded platelet count	Day of hematologic recovery†
2.5	13	2.8 (1.8-11.1)	1.0 (0.1-8.5)	16 (3-22)	150 (79-307)	13 (3-28)	23 (20-33)
2.0	6	2.3 (1.2-4.8)	1.25 (0.1-2.8)	16 (17-21)	124 (18-418)	13 (11-25)	22 (15-28)

*All values given are medians with ranges in parentheses.

†Granulocyte count > 1.5 cells × 10^3/mm^3; platelet count > 100 cells × 10^3/mm^3.

four because of excessive toxicity (hematologic in two) during the first course. There were no drug-related deaths.

There was no evidence of toxicity in the one patient treated at the 1.25-mg/m^2 level because of abnormal liver function tests.

DISCUSSION

The long plasma and tissue half-life of actinomycin D demonstrated in experimental animals was the primary pharmacologic reason for this clinical study (5). Although we have not yet studied our patients pharmacologically, clinical pharmacologic data on actinomycin D did become available after our study was initiated which confirmed a long plasma half-life of the drug in man of approximately 36 hours (6). An intermittent dosage schedule for adriamycin based on its similar pharmacokinetic behavior has increased the therapeutic index of that drug (7).

More recently, two additional observations in experimental animals have added extra support to the use of an intermittent single-dose schedule of actinomycin D. The first was a demonstration by Schabel that a single nonlethal dose of actinomycin D was curative in mice bearing Ridgway osteogenic sarcoma at a stage where repeated daily doses for 7 days were not curative despite a total dose equal to the LD10 for normal mice (8). Subsequently, Galbraith and his associates have demonstrated higher peak concentrations of actinomycin D in tumor tissue when the drug was given in single high doses rather than in multiple low doses, and they have suggested that antitumor effect may be proportional to peak drug concentration (9).

Recent toxicologic data in beagle dogs have been interpreted to show that actinomycin D was more toxic when given as a single dose as opposed to divided daily doses (10). The studies reported were in conflict with previous studies (11), concentrated on lethal toxicity, and did not take into account the therapeutic index of the drug. Our clinical data do not support the contention that actinomycin D is more toxic when given as a single dose. In contrast, at our recommended dose level of 2 mg/m^2, the toxicity is less than that usually observed with a "standard" loading-dose schedule of 0.5 mg (maximal dose) daily for 5 days despite the fact that the total dose delivered is considerably higher. The dose-limiting toxicity appears to be mucositis primarily confined to the oral mucosa, although one of our patients at the 2.5-mg/m^2 dose had documented gastric ulcerations. The other dose-limiting toxic effect seen only at the 2.5-mg/m^2 dose was severe dermatitis, often exacerbated by radiation. This was not seen in the patients treated with the 2-mg/m^2 dose and did not appear to be related to cumulative dose if an interval of at least 4 weeks was maintained between doses.

The 9% complete and partial remission rate observed in our patients with malignant melanoma is particularly gratifying. Although disease was limited to soft tissue in the patient who had a complete remission, the patient with partial remission had a > 90% shrinkage of a mediastinal mass, complete resolution of a pleural effusion, and clearing of malignant cells from his bone marrow. An additional 27% of the patients in this category had stabilization of previously progressive disease. Such a finding is usually indicative of a drug effect. Since patients with melanoma refractory to DTIC rarely respond to other chemotherapy except, on occasion, nitrosoureas, the responses seen here in patients often receiving actinomycin D as third- or fourth-line therapy are all the more impressive. Further trials with single-dose actinomycin D in patients with malignant melanoma are certainly warranted at an earlier stage and in conjunction with immunotherapy and perhaps other chemotherapy. Phase II trials of this drug in other tumors may also be indicated at the 2-mg/m^2 dose every 4 weeks. Such studies would be of particular interest in pediatric patients, considering the importance of actinomycin D in the treatment of childhood tumors.

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Neurotoxicity From 5-Fluorouracil (NSC-19893) Administration Reproduced by Mitomycin C (NSC-26980)[1,2]

Brief Reports and Preliminary Communications

Michael Nichols, Patrick R. Bergevin,* Avinash C. Vyas, and Robert Hamlin[3]

The following is a case report of unique neurotoxicity associated with 5-fluorouracil (5-FU) treatment and reproduced by later therapy with mitomycin C. Various etiologic considerations are discussed.

CASE REPORT

A 65-year-old Caucasian man was admitted to Coney Island Hospital in November 1973 with abdominal pain and chronic constipation. A barium enema revealed an annular filling defect involving the sigmoid colon, and biopsy of this lesion disclosed an infiltrating adenocarcinoma. He underwent a laparotomy, at which time diffuse liver metastases were seen. A palliative resection of the colonic tumor with end-to-end anastomosis was performed.

In July 1974 therapy was started with weekly 5-FU at a dose of 15 mg/kg given orally or iv. Before treatment was started a neurologic examination was done and revealed no abnormalities and there was no past history of neurologic abnormalities. After eight doses of 5-FU the patient developed various neurologic abnormalities including ataxia, episodes of dizziness, masked facies and a parkinsonian gait, severe generalized muscular weakness (most pronounced in the proximal muscle groups of the lower extremities) with rigidity, and a fine generalized tremor. The 5-FU was discontinued and the patient noted improvement in neurologic dysfunction after 2 weeks; complete resolution of symptoms and signs had occurred by 4 weeks. The results of a brain scan taken at this time were normal. 5-FU was resumed at a lower dose of 500 mg weekly iv, but after two doses of the drug the identical neurotoxicity returned accompanied by a marked lability of mood and impairment in concentration. The drug was again discontinued and within 4 weeks the patient had completely recovered. At this time the results of the neurologic examination were normal. A lumbar puncture was performed and cerebrospinal fluid pressure and chemistries were normal.

In January 1975 the patient was treated with mitomycin C at a dose of 2.5 mg weekly iv, but after two doses he began to complain of a severe aching pain in the lower extremities with generalized weakness, again most pronounced in the lower extremities. Within a few days the same neurologic manifestations associated with the 5-FU therapy returned and the mitomycin C was stopped. Within 4 weeks there was complete improvement in neurologic dysfunction, and a detailed neurologic examination done at that time was unremarkable except for a mild lability of mood and a fine generalized tremor of the extremities.

The patient has since received no further chemotherapy. In April 1975 he received a course of radiotherapy to a recurrent adenocarcinoma in the anastomotic site which was bleeding and causing pain; there was no progressive neurologic abnormality noted during or after the radiotherapy. A repeat brain scan at this time was also within normal limits.

DISCUSSION

An acute cerebellar syndrome has been described as a neurotoxic manifestation of 5-FU, which may consist of gross dysmetria, slurred speech, ataxia of trunk or extremities, coarse nystagmus, and dizziness (1). It has been estimated that < 1% of patients receiving 5-FU will develop manifestations of cerebellar dysfunction, although the incidence apparently increases when high doses or intensive daily loading regimens of the drug are used (2). The syndrome is almost always reversible within 6 weeks after discontinuation of therapy and may not return even though the drug is resumed at the same dose and interval between courses (3). It is known that 5-FU crosses the blood-brain barrier after iv injection, and in mice it has been detected in higher concentrations in the cerebellum than in other parts of the brain (4). The precise cause of the cerebellar dysfunction, however, is not known.

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[2]5-Fluorouracil: CAS reg. No. 51-21-8.
Mitomycin C: CAS reg. No. 50-07-7.

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In the patient described in this report, a unique neurologic dysfunction developed during 5-FU therapy consisting of cerebellar and basal ganglia abnormalities which resolved after discontinuation of the drug but recurred when the patient was subsequently re-challenged with a lower dose of 5-FU. It was then surprising to see the identical neurologic dysfunction soon after the start of chemotherapy with mitomycin C, again with resolution after discontinuation of the drug. To the best of our knowledge, mitomycin C has not been demonstrated to cross the blood-brain barrier after iv injection and has not previously been associated with neurologic abnormalities.

Repeat brain scans did not disclose evidence of brain metastases, and the quick reversal of the neurologic dysfunction upon discontinuation of the 5-FU or mitomycin C makes this possibility unlikely.

Another etiologic possibility stems from the recent report of a patient with a pre-existing neuropathy who suffered a heightened and fatal neurotoxic reaction to vincristine (5). Paraneoplastic cerebral and cerebellar cortical degeneration may occur with malignancies not directly invading the central nervous system. It is postulated but cannot be proven that chemotherapeutic agents with a neurotoxic potential may cause a worsening of or even unmask such paraneoplastic states.

Finally, tumor breakdown by chemotherapy and liberation of substances which are neurotoxic, either by direct action on the central nervous system or by an autoimmune mechanism, are also possible considerations in this case.

These etiologic considerations are mentioned because of (*a*) the absence of prior reports of neurotoxicity associated with mitomycin C, and (*b*) the fact that two structurally different agents with supposedly different mechanisms of action have seemingly produced identical and reversible neurologic syndromes.

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Staging Colorectal Cancer: Is There a Role for Liver Biopsy?[1,2]

Brief Reports and Preliminary Communications

Barry Rosenbloom, Jerome B. Block,* and Yosef Pilch[3]

Recently, attention has been focused on colorectal cancer as potentially yielding to therapeutic combinations of agents which are weakly antineoplastic when used singly against colon cancer (1,2). Full assessment of patient survival in studies using this approach may not be possible for years. Evaluation of these data will in part depend on how well patients have been clinically staged or stratified for study on the basis of known biologic determinants of survival in colorectal cancer. While we now know and use the important variable of Duke's classification, it is the purpose of this communication to suggest that other variables may be at hand which may at least equally influence survival and response to therapy.

One of the important recent developments in clinical staging of cancer has been the awareness that the usual clinical and laboratory assessments for hepatic involvement by malignant lymphoma are often inadequate to detect biopsy-provable liver metastatic disease (3,4). Such observations have led to more complete staging of these diseases and, consequently, to more reliable assessment of the efficacy of therapies being compared. Many clinical research programs now utilize peritoneoscopy, laparotomy, or both with multiple needle biopsies of the liver to adequately stage the extent of lymphoma. The estimates of hepatic involvement by Hodgkin's disease at the time of clinical presentation range from 5% (5) by closed biopsy to 20% at laparotomy (3). On the other hand, in non-Hodgkin's lymphoma the incidence of hepatic involvement at the time of diagnosis has ranged from 16% (4) to 40% (6).

The incidence of hepatic metastases at the time of diagnosis in patients with colorectal carcinoma has been reported at 16% (7) and 19% (8) in two separate studies. On the other hand, 64% (9) of the patients initially determined to be "curable" at resection had liver metastases at autopsy. In three large series (9-11) the specific incidence of liver metastases in colorectal cancer is not recorded, but of the 18%-27% of patients with Duke's D disease the majority were said to have liver metastases. The method used to determine this was mainly direct inspection of the liver at the time of operation; however, correlation with liver scans and/or liver function tests is not recorded.

Studies of metastatic solid tumors to the liver in general reveal an incidence of 10%-14% (12,13) in false-negative results by isotopic imaging. Liver function studies have shown relatively good correlation in metastatic solid tumors (14), yet 15% of the patients with lesions discovered at laparotomy had normal levels of alkaline phosphatase. In Hodgkin's disease, the alkaline phosphatase level is less useful because even in the absence of hepatic involvement, significant numbers of patients have elevated alkaline phosphatase (15). More important prognostically is that patients who have Duke's D colorectal cancer at the time of diagnosis have a 3-7–month median survival (9-11) and it is clear that liver metastases play a significant role in this poor outcome. The question that would logically follow is whether the high incidence of liver metastases at postmortem examination is related to inadequate diagnostic methodology at the time of laparotomy. Alternatively, does hepatic metastatic disease occur after the initial diagnostic and surgical manipulations and from residual and local recurrent malignant disease? Are current techniques of scanning, liver function studies, and direct inspection with palpation at surgery adequate in assessing the initial true incidence of hepatic metastases in colorectal cancer? The question has not yet been answered by attempts at systematic liver biopsy at the time of primary resection. Utilization of liver biopsy by the percutaneous route may not be useful in metastatic solid tumors such as colorectal cancer. Postmortem "blind" percutaneous liver biopsies in the presence of known hepatic metastases have yielded a 50% (16) incidence of positivity after two biopsies; it can thus be argued that since solid tumor metastases are typically discrete nodules it is not surprising that they may be missed by a needle biopsy.

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It is our suggestion that even in the absence of visible or palpable nodules at the time of surgery, "blind" bilobular needle biopsies of the liver at the time of primary tumor resection may prove valuable in staging patients more accurately and the area is worthy of study. The morbidity from intraoperative needle biopsy is minimal and the questions regarding the true "curability" of resected lesions can be more adequately determined. Perhaps 10%-20% of hepatic metastases not discovered by other modalities might be detected. These biopsies could be done safely at the start of intra-abdominal surgery prior to any handling of the tumor and the biopsy sites could be re-examined subsequently for hemorrhage prior to closure. The 5-year survival of Duke's A lesions ranges from 60% to 80% (9-11), possibly implying a major defect in initial staging. This may be due to occult but detectable hepatic metastatic disease.

In summary, we feel that utilizing a research approach which includes blind biopsies of each hepatic lobe intraoperatively may yield new and useful data affecting the prognosis of patients with colorectal cancer. An additional outcome of such an approach might be more reliable data on assessing response and survival in therapeutic research programs for colorectal cancer.

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ANNOUNCEMENT

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